The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

VOLUME 19 APRIL, 1959 NUMBER 4
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FURTHER STUDIES OF 21-DEOXYHYDROCORTISONE-4-C^{14} IN MAN*

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ABSTRACT

In a study of the metabolism of 21-deoxyhydrocortisone-4-C^{14} in a normal subject and a patient with congenital adrenal hyperplasia it was found that there was no marked difference in the metabolism of the "abnormal" hormone in these 2 subjects. The principal metabolite of 21-deoxyhydrocortisone was 11-ketopregnane-3α,17α,20α-triol, which comprised 44 to 51 per cent of the total steroid radioactivity. Pregnane-3α,11β,17α,20α-tetrol and 3α,17α-dihydroxypregnane-11,20-dione each accounted for about 10 per cent of the total radioactivity. Another 8 per cent of the metabolites consisted of pregnane-3α,11β,17α,20β-tetrol, 11-ketopregnane-3α,17α,20β-triol and 20-ketopregnane-3α,11β,17α-triol. Cleavage of the side chain to form 11-oxygenated 17-ketosteroids was only a minor transformation of 21-deoxyhydrocortisone. This result is interpreted to mean that the large amounts of 11-oxygenated C_{19} steroid metabolites usually present in the urine of patients with congenital adrenal hyperplasia are derived principally from C_{19} hormonal precursors. The daily production of 21-deoxyhydrocortisone was calculated to be between 37 mg. and 66 mg. in the patient with adrenal hyperplasia.

IT IS well established that one type of congenital adrenal hyperplasia (CAH) in man is defined in biochemical terms as a relative inability to oxygenate carbon 21 to form the hydrocortisone molecule (1–3). This lesion results in the secretion of 21-deoxyhydrocortisone (I), recognized

Received for publication November 1, 1958.

* This investigation was supported in part by a grant from the American Cancer Society and a research grant (CY-3207) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

from the urinary steroids, 11-ketopregnane-3α,17α,20α-triol (VI) (3–5) and pregnane-3α,11β,17α,20α-tetrol (III) (3) (Fig. 1). Together with these compounds, which are not present in detectable amounts in normal urine, there is a greatly increased production of the conventional 17-ketosteroids, of both the 11-oxygenated and the 11-deoxy type (2, 3, 6–11). The present investigation was primarily designed to obtain information as to the extent of side-chain cleavage of 21-deoxyhydrocortisone, in order to determine whether this abnormal secretory product could serve as the precursor of the 11-oxygenated 17-ketosteroids. In addition it was of interest to establish whether there was any gross difference in the total metabolic pattern of the normal human subject as compared with the CAH patient.

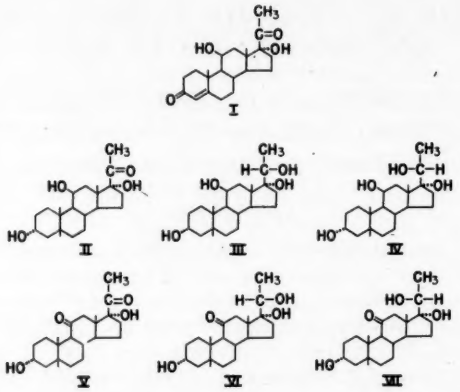

FIGURE 1

With these aims in view, the metabolism of 21-deoxyhydrocortisone-4-C^{14} was critically examined in a normal subject. It was shown by the rigorous technique of isotopic dilution that the major portion of 21-deoxyhydrocortisone was recovered in the urine as 3 metabolites still containing the 21-carbon skeleton. These were pregnane-3α,11β,17α,20α-tetrol (III), 11-ketopregnane-3α,17α,20α-triol (VI) and 3α,17α-dihydroxypregnane-11, 20-dione (V). By the same precise method it was demonstrated that 2 of the principal 11-oxygenated 17-ketosteroids, namely 3α, 11β-dihydroxyetiocholane-17-one and 3α,11β-dihydroxyandrostane-17-one, were produced in only minor amounts from the radioactive precursor steroid.

Since these facts appeared to be well established in the normal subject, 21-deoxyhydrocortisone-4-C^{14} was administered to a patient with untreated CAH who had been extensively studied in these laboratories. By the technique of paper chromatography in several systems it was shown that the major metabolites of the steroid were the same in this patient as in the

normal subject, and that the metabolic pattern was similar. Moreover it was found in the patient with CAH, just as in the normal subject, that only a negligible portion of the 21-deoxysteroid was converted to 17-ketosteroids. It is evident from the data in Table 2 that there is reasonably good agreement between estimates based on paper chromatography alone and the more exact measurements based on reverse isotopic dilution. Since a more accurate definition of the precise amount of each individual metabolite would hardly contribute to the general problem, major emphasis by the technique of reverse isotopic dilution was devoted to an analysis of 2 other potential end-products, pregnane-3α,11β,17α,20β-tetrol (IV) and 11-ketopregnane-3α,17α,20β-triol (VII). These 20β isomers of 2 of the principal metabolites of 21-deoxyhydrocortisone were, as anticipated, present in the urine but the amounts were considerably less than those of the corresponding 20α epimers. In addition, another metabolite, 20-ketopregnane-3α,11β,17α-triol (II) was shown to contain a small portion of the total radioactivity derived from the precursor steroid. With the evaluation of these 6 metabolites, the principal aspect of the metabolism of a "defective" hydrocortisone molecule was evident.

EXPERIMENTAL[1]

Methods

Paper chromatography: The paper chromatography was carried out at 24° C. on 18 cm.×57 cm. strips of Whatman No. 1 paper. The mobilities of the 21-deoxyhydrocortisone metabolites are recorded in Table 1.

Radioactivity assay: Samples were counted as infinitely thin solid mounts in a Tracer-Lab Model 50 automatic sample changer. The paper chromatograms were scanned in an Aqueboque chromatogram scanner coupled with a Nuclear Model 1620 rate meter and a Brown mv. recorder. Depending on the amount of radioactivity present, the scanning time varied between one and four hours, the longer time being required for samples of low radioactivity. After the scanning was completed, appropriate areas of the chromatograms were eluted and the extracts were counted.

Patient with congenital adrenal hyperplasia

A dose of approximately 5 mg. of 21-deoxyhydrocortisone-4-C^{14} containing 1,600,000 c.p.m.[2] in 123 Gm. of 5 per cent glucose solution and a small amount of ethanol was administered intravenously over a period of thirty minutes to a patient with congenital adrenal hyperplasia.[3]

During the first twenty-four hours after the dose, 1,400,000 c.p.m. or 90 per cent of the dose was excreted in the urine. The urinary conjugates were

[1] All melting points are corrected.

[2] The labeled 21-deoxyhydrocortisone was obtained from the Endocrinology Study Section of the United States Public Health Service.

[3] The daily excretion of urinary steroid metabolites of this subject has previously been reported (3).

TABLE 1. PAPER CHROMATOGRAPHY OF THE METABOLITES OF 21-DEOXYHYDROCORTISONE

System*	Compound (50 μg.)	Mobility (cm. from origin)
"A" 20 hrs.		
Toluene-	Pregnane-3α,11β,17α,20α-tetrol(III)	8–11
Isooctane 3:1	Pregnane-3α,11β,17α,20β-tetrol(IV)	14–19
Methanol-	11-Ketopregnane-3α,17α,20α-triol(VI)	21–28
Water 4:1	20-Ketopregnane-3α,11β,17α-triol(II)	32–36
	11-Ketopregnane-3α,17α,20β-triol(VII)	34–38
"B" 3 hrs.		
Toluene-	3-Monoacetate of II	33–38
Isooctane 5:3	3,20-Diacetate of VII	39–44
Methanol-		
Water 4:1		
"C" 32 hrs.		
Toluene-	3α,17α-Dihydroxypregnane-11,20-dione(V)	9–12
Propylene glycol	3α,11β-Dihydroxyetiocholane-17-one	22–26
	3α,11β-Dihydroxyandrostane-17-one	33–37
"D" 32 hrs.		
Cyclohexane-	3α-Hydroxyetiocholane-11,17-dione	23–27
Toluene 1:1	3α-Hydroxyandrostane-11,17-dione	33–37
Propylene glycol		

* Systems A and B are equilibrated by shaking together equal volumes of two phases at 24° C.

hydrolyzed with β-glucuronidase[4] at pH 5.0 for five days at 37° C. The urine was then adjusted to pH 1 with sulfuric acid and continuously extracted with ether for forty-eight hours. The neutral steroid fraction prepared in the usual way contained 1,030,000 c.p.m.

Isolation of metabolites by reverse isotopic dilution. To a portion (82,400 c.p.m.) of the neutral steroid extract were added 30.4 mg. of pregnane-3α, 11β,17α,20β-tetrol (IV) and 30.1 mg. of 11-ketopregnane-3α,17α,20β-triol (VII). The mixture was chromatographed on 40 Gm. of silica gel containing 16 ml. of ethanol. The column was started with 3 per cent ethanol in chloroform.

11-Ketopregnane-3α,17α,20β-triol (VII). Elution with 7 per cent ethanol in chloroform afforded 11-ketopregnane-3α,17α,20β-triol, which was judged to be uncontaminated by other metabolites by chromatography on paper in System A. Two recrystalli-

[4] The enzyme, β-glucuronidase, used for hydrolysis was obtained from the Warner-Chilcott Laboratories, a division of Warner-Lambert Pharmaceutical Company, New York; it is commercially available under the trade name Ketodase

zations from ethyl acetate yielded VII, m.p. 218–219°, 130 c.p.m. per mg. After a subsequent recrystallization from acetone the specific activity was 133 c.p.m. per mg. The remaining crystals were acetylated and recrystallized from methanol, 101 c.p.m. per mg. equivalent to 126 c.p.m. per mg. for the free steroid. The total radioactivity of VII was therefore 3,800 c.p.m. or 4.6 per cent of the neutral steroid fraction.

In order to insure that this product was uncontaminated with 11-ketopregnane-3α,17α,20α-triol (VI) the mother liquors of the first 2 recrystallizations of the free steroid were combined and chromatographed on several papers with System A. The areas corresponding in mobility to 11-ketopregnane-3α,17α,20β-triol were eluted and care was taken to exclude areas which might have contained the 20α isomer. In this fashion 9 mg. were obtained and after recrystallization the specific activity was 126 c.p.m. per mg. In view of the close agreement with the previous result, no further purification seemed necessary.

Pregnane-3α,11β,17α,20β-tetrol (IV). Further elution of the silica gel partition column with 7 per cent ethanol in chloroform gave 29 mg. of pregnane-3α,11β,17α,20β-tetrol uncontaminated by other metabolites as judged by paper chromatography. Two recrystallizations from methanol yielded IV, m.p. 276–278°, 19 c.p.m. per mg. After a subsequent recrystallization from ethanol the specific activity was 22 c.p.m. per mg. The total radioactivity of IV thus was 600 c.p.m. or 0.7 per cent of the neutral steroid extract.

20-Ketopregnane-3α,11β,17α-triol (II). A 10 per cent aliquot (103,000 c.p.m.) of the neutral steroid extract was added to 30.1 mg. of 20-ketopregnane-3α,11β,17α-triol (II) and the mixture was chromatographed on 40 Gm. of silica gel containing 16 ml. of ethanol. The column was started with 3 per cent ethanol in methylene chloride. Elution with 7 per cent ethanol in methylene chloride afforded fractions containing II, as judged by paper chromatography. These fractions were combined, acetylated (36 mg.) and chromatographed on 40 Gm. of silica gel containing 16 ml. of *tert*-butyl alcohol. A small amount of endogenous 11-ketopregnane-3α,17α,20α-triol 3,20-diacetate was eluted with 2 per cent *tert*-butyl alcohol in methylene chloride. Elution with 3 per cent *tert*-butyl alcohol in methylene chloride afforded 20-ketopregnane-3α,11β,17α-triol 3-monoacetate. The fractions containing only the monoacetate of II, as judged by paper chromatography, were combined (5 mg.). Recrystallization from methanol yielded 20-ketopregnane-3α,11β,17α-triol 3-monoacetate, 85 c.p.m. per mg. There was insufficient material for further purification. The specific activity of the acetate corresponded to 95 c.p.m. per mg. of the free steroid or a total radioactivity in II of 2900 c.p.m. This represented 2.8 per cent of the neutral steroid extract.

Fractionation of 21-deoxyhydrocortisone-4-C^{14} metabolites by paper chromatography. Ten per cent of the neutral extract (103,000 c.p.m.) was streaked across the central 5 cm. of the starting line on a sheet of Whatman No. 1 paper (18 cm.$\times$57 cm.). A mixture containing 50 μg. of each of the pure reference steroids was placed on spots about 3.5 cm. removed from the radioactive extract and about 2.5 cm. from either edge of the paper. Using System A, the chromatogram was then developed for thirty hours and the paper was dried. Five cm. strips from either edge of the paper containing the reference steroids were cut out and stained with phosphomolybdic acid. From the remainder of the chromatogram, a center strip of about 3.5 cm. in width was cut and counted in the strip counter. Using the stained reference steroids and the plot of radioactivity from the Brown recorder as guides, the whole paper (excluding the outer edges which contained the reference steroids) was cut into sections corresponding to the areas where radioactivity was present. There were 4 well defined zones of radioactivity as shown in Figure 2. In order of increasing polarity these corresponded respectively to a mixture of 20-ketopregnane-3α,11β,17α-triol (II) and 11-ketopregnane-3α,17α,20β-triol (VII). 11-

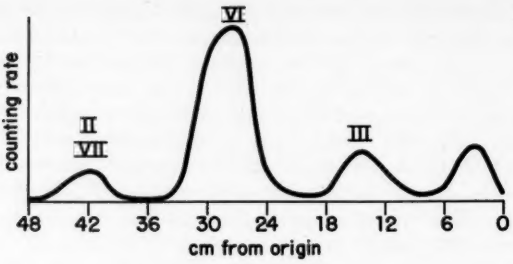

FIG. 2. Paper chromatogram (System A) of the radioactive metabolites of 21-deoxyhydrocortisone.
II = 20-Ketopregnane-3α,11β,17α-triol.
III = Pregnane-3α,11β,17α,20α-tetrol.
VI = 11-Ketopregnane-3α,17α,20α-triol.
VII = 11-Ketopregnane-3α,17α,20β-triol.

ketopregnane-3α,17α,20α-triol (VI), pregnane-3α,11β,17α,20α-tetrol (III) and another zone only slightly removed from the origin. These were eluted with methanol-chloroform, 1:1, and the 4 areas were counted. Since 11-ketopregnane-3α,17α,20β-triol (VII) and 20-ketopregnane-3α,11β,17α-triol (II) have the same mobility in System A, the least polar area which contained this mixture (6.1 per cent of the radioactivity in the neutral extract) was acetylated. Rechromatography of the acetates in System B separated 3α,20β-diacetoxy-17α-hydroxypregnane-11-one from 3α-acetoxy-11β,17α-dihydroxypregnane-20-one.

The effluent from System A was rechromatographed with System C in order to separate 3α,17α-dihydroxypregnane-11,20-dione (V), 3α,11β-dihydroxyetiocholane-17-one and 3α,11β-dihydroxyandrostane-17-one. The effluent from System C was in turn rechromatographed with System D to separate 3α-hydroxyetiocholane-11,17-dione and 3α-hydroxyandrostane-11,17-dione. The results of these chromatographic separations are recorded in Table 2.

Normal subject

Some details of this study, including the recovery of radioactivity in the urine (73 per cent of the dose), hydrolysis, fractionation and isolation of 11-ketopregnane-3α,17α,20α-triol (44 per cent of the neutral fraction after enzymatic hydrolysis) have been described (12).

After removal of most of the 11-ketopregnane-3α,17α,20α-triol (VI) with its attendant radioactivity, all the mother liquors from the first crystallizations were added to the chromatographic fractions of both the ketonic and non-ketonic portion of this extract. The mixture so obtained had 196,000 c.p.m. and represented 60 per cent of the total radioactivity of the neutral steroid fraction. To this combined mixture the following steroids were added as carriers: 40.0 mg. of 3α,17α-dihydroxypregnane-11,20-dione (V); 30.0 mg. of 3α,11β-dihydroxyetiocholane-17-one; 20.0 mg. of 3α,11β-dihydroxyandrostane-17-one; and 40.0 mg. of pregnane-3α,11β,17α,20α-

TABLE 2. URINARY STEROIDS DURING TWENTY-FOUR HOURS FOLLOWING INTRAVENOUS ADMINISTRATION OF 21-DEOXYHYDROCORTISONE-4-C^{14}

	Per cent of neutral steroid extract		
	Adrenal hyperplasia		Normal subject
	PC*	RID†	RID†
Pregnane-3α,11β,17α,20α-tetrol(III)	9.3		9.8
Pregnane-3α,11β,17α, 20β-tetrol(IV)		0.7	
11-Ketopregnane-3α,17α,20α-triol(VI)	51.0		44.3
11-Ketopregnane-3α,17α,20β-triol(VII)	3.6	4.6	
20-Ketopregnane-3α,11β,17α-triol(II)	2.5	2.8	
3α,17α-Dihydroxypregnane-11,20-dione(V)	7.0		12.2
3α,11β-Dihydroxyetiocholane-17-one	0.4		0.3
3α,11β-Dihydroxyandrostane-17-one	0.3		0.1
3α-Hydroxyetiocholane-11,17-dione	0.03		
3α-Hydroxyandrostane-11,17-dione	0.2		

* PC = paper chromatography.
† RID = reverse isotopic dilution.

tetrol (III). The mixture of extract and carrier steroids was separated into ketonic (72,000 c.p.m.) and non-ketonic (107,000 c.p.m.) steroid fractions by means of Girard's reagent T.

3α,11β-Dihydroxyandrostane-17-one. The ketonic fraction (127 mg.) was chromatographed on 80 Gm. of silica gel containing 32 ml. of ethanol. Elution with 2 per cent ethanol in methylene chloride yielded 10 mg. of 3α,11β-dihydroxyandrostane-17-one uncontaminated by other metabolites, as judged by paper chromatography. After 2 recrystallizations from ethyl acetate, the m.p. of the steroid was 198–200° and specific activity 15 c.p.m. per mg. Further recrystallization did not alter the specific activity. The total radioactivity, 300 c.p.m., contained in 3α,11β-dihydroxyandrostane-17-one, represented 0.1 per cent of the neutral steroid extract.

3α,11β-Dihydroxyetiocholane-17-one. Further elution of the column with the same solvent yielded 17 mg. of 3α,11β-dihydroxyetiocholane-17-one uncontaminated with other steroids, as judged by paper chromatography. After 2 recrystallizations from methanol-ethyl acetate, the m.p. of the steroid was 237.5–238.5°, and specific activity 29 c.p.m. per mg. After recrystallization from acetone, the specific activity was 26 c.p.m. per mg. Acetylation and recrystallization yielded 3α-acetoxy-11β-hydroxyetiocholane-17-one, m.p. 236–237°, 28 c.p.m. per mg., corresponding to 32 c.p.m. per mg. as the free steroid. The average specific activity was 28 c.p.m. per mg., or a total radioactivity content of 840 c.p.m.; this represented 0.25 per cent of the neutral steroid extract.

3α,17α-Dihydroxypregnane-11,20-dione (V). Elution of the partition column with 4 per cent ethanol in methylene chloride afforded 18 mg. of 3α,17α-dihydroxypregnane-11,20-dione (V), as judged by paper chromatography and infrared spectrometry. Recrystallization from ethyl acetate and acetone yielded V, m.p. 190.5–193°, and specific activity 990 c.p.m. per mg. A subsequent recrystallization from ethyl acetate had a spe-

cific activity of 995 c.p.m. per mg. Acetylation and recrystallization from methanol yielded 3α-acetoxy-17α-hydroxypregnane-11,20-dione, m.p. 193–196°, and specific activity 910 c.p.m. per mg., corresponding to 1020 c.p.m. per mg. for the free steroid. The average specific activity was 1000 c.p.m. per mg. or a total radioactivity in 3α,17α-dihydroxypregnane-11,20-dione of 40,000 c.p.m. This represented 12.2 per cent of the neutral steroid extract.

Pregnane-3α,11β,17α,20α-tetrol (III). The non-ketonic fraction (208 mg.) was chromatographed on 40 Gm. of silica gel containing 16 ml. of ethanol. Elution was started with 1 per cent ethanol in chloroform and continued with increasing proportions of ethanol until 41 mg. of pregnane-3α,11β,17α,20α-tetrol was obtained with 9 per cent ethanol in chloroform. This product was virtually pure, as judged by paper chromatography and infrared spectrometry. Recrystallizations from ethyl acetate-benzene yielded III, with specific activities of 820 and 810 c.p.m. per mg. Acetylation and recrystallization from methanol yielded pregnanetetrol-3,20-diacetate, m.p. 220–222°, and specific activity 680 c.p.m. per mg., corresponding to 830 c.p.m. per mg. for the free steroid. The average specific activity was 820 c.p.m. per mg., or a total radioactivity of 32,800 c.p.m. in pregnane-3α,11β,17α,20α-tetrol. This represented 10 per cent of the neutral extract.

DISCUSSION

Since the completely oxygenated side chain of hydrocortisone can normally give rise to 11-oxygenated 17-ketosteroids by oxidative removal of 2 carbon atoms, the question arose whether 21-deoxyhydrocortisone serves a similar precursor role for the 11-oxygenated 17-ketosteroids of patients with CAH. The amount of 17-ketosteroid derived from 21-deoxyhydrocortisone was so small that this precursor could never account for the levels of 11-oxygenated 17-ketosteroids in CAH. The results of the present investigation, therefore, strongly imply that these metabolites, for the most part, are derived from C_{19} hormonal precursors directly and are not the result of metabolic breakdown of a 21-carbon steroid. This conclusion agrees with other evidence, since the adrenal glands of man as well as of the lower animals can produce C_{19} steroid hormones. If it be assumed that the rate of steroid production per unit weight is at a normal level for a smaller organ, the fact that the adrenals are enlarged would account for the increased production of C_{19} metabolites because of the high corticotropin levels in this disorder (13).

The present investigation likewise supports the view that there is no marked difference in extra-adrenal steroid metabolism between a normal subject and a patient with CAH. The rather close correspondence of the metabolites of 21-deoxyhydrocortisone in these 2 subjects agrees with previous evidence that no disturbance of hydrocortisone metabolism is evident in CAH (14). This same conclusion was reached by Finkelstein and Goldberg (15) on the basis of quite different experiments.

Including the information this study provides about CAH, some generalizations are possible about the biochemical transformation of adrenal steroids in man. In particular, it is of interest that a single metabolite was

the major transformation product of 21-deoxyhydrocortisone. This may well be true with many other 21-deoxysteroids, since the evidence indicates that 17-hydroxyprogesterone is predominantly transformed to pregnane-3α,17α,20α-triol, and progesterone yields mainly pregnane-3α,20α-diol. In this sense these 21-deoxy hormones resemble 11β-hydroxy-Δ^4-androstene-3,17-dione, which affords 1 principal metabolite, and contrast with both hydrocortisone, which yields an array of end-products, and testosterone, which is converted chiefly to 2 compounds. There is another point of similarity in the metabolism of 21-deoxy hormones, in that a 20α-oriented hydroxyl group is the prominent form, and the corresponding 20β-epimer and 20-ketosteroids are a smaller fraction.

The metabolites III and VI are formally related to cortol and cortolone, whereas the 20-ketosteroids V and II may be considered the 21-deoxy counterparts of "tetrahydro E" and "tetrahydro F." The artifacts derived from III and VI, 3α-hydroxypregnane-11,20-dione and 3α-hydroxy-$\Delta^{9[11]}$-pregnene-20-one, were previously isolated (16) in small amounts (2–3 per cent and 2–6 per cent respectively) after administration of unlabeled 21-deoxyhydrocortisone. Earlier, 3α,17α-dihydroxypregnane-11,20-dione (V) was shown to be a metabolite of 21-deoxycortisone (0.4 per cent yield) (17), and the 11-hydroxy analogue II was isolated in about 3–8 per cent yield from 21-deoxyhydrocortisone as the artifact of acid hydrolysis, 3α,17α-dihydroxy-$\Delta^{9[11]}$-pregnene-20-one (16). It is of interest that in the isotopic experiments the end-products of metabolism of the 21-deoxysteroid were principally 11-ketones. This was found to be true when the 20-hydroxyl group was α or β oriented as well as when there was a carbonyl group at C-20. It is noteworthy also that the ratio 11-keto:11-hydroxy in the metabolites of the administered radioactive steroid was very similar to that found in the daily endogenous production of III and VI by the CAH patient under basal conditions (3).

From a knowledge of the endogenous steroid production of this CAH patient and the metabolism of 21-deoxyhydrocortisone, it is possible to calculate a reasonable approximation of his daily secretion of this "defective" hormone. A 24-hour specimen of his urine yielded by isolation 12 mg. of 11-ketopregnane-3α,17α,20α-triol (VI) and 4 mg. of the corresponding tetrol (III). In the tracer study, these same products were respectively 51 per cent and 9.3 per cent of the neutral steroid extract, or 33.5 per cent and 6 per cent respectively of the dose administered. From these values it is evident that his adrenal glands secreted between 37 and 66 mg. per day of 21-deoxyhydrocortisone, with a strong probability that the higher value is the more accurate approximation. This estimate amply confirms the view that endogenous production of this unusual "hormone" was of considerable magnitude in the patient with CAH.

Acknowledgment

The authors wish to express their appreciation to their colleague, Dr. Barnett Zumoff, for his invaluable cooperation. The technical assistance of Mrs. Su-hsia Hong, Miss Nella Hellinger and Miss Ruth Jandorek is gratefully acknowledged.

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METABOLIC STUDIES IN PREGNANCY AND THE PUERPERIUM*

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ABSTRACT

Metabolic balance studies, determinations of body fluid compartments and measurement of urinary corticosteroid excretion were carried out in 5 pregnant women during the latter part of gestation and immediately post partum. During the study significant storage of nitrogen and potassium and expansion of extracellular volume occurred in the antepartum period. The accelerated weight loss following delivery may have been in part associated with the restoration of body fluid compartments to normal. Negative nitrogen and potassium balances were observed at this time, associated with a rise in the levels of serum nonprotein nitrogen and potassium. Sodium storage was most marked in the antepartum period, and continued to a lesser degree in the postpartum period when the overall sodium balance was considered for this part of the study. Sodium diuresis was always observed soon after delivery. The establishment of a new metabolic equilibrium had not occurred by the time of cessation of these studies. In the antepartum period there was an apparent correlation between sodium retention and aldosterone excretion. Immediately post partum, variations in sodium excretion were also associated with changes in aldosterone excretion, but later in the postpartum period aldosterone excretion returned to nonpregnant levels and did not vary with further changes in the sodium balance. An increase in total blood volume, extracellular fluid volume, plasma volume, and total body water was observed in the antepartum period. There was a postpartum reduction in these parameters, and the data suggest that it may have been partly due to the alterations in aldosterone secretion.

AMONG the many metabolic changes occurring during pregnancy, the retention of fluid and sodium has received particular attention. By means of a variety of methods, a progressive increase in blood volume, total body water and extracellular and intracellular fluid spaces has been observed by many investigators (1–10). Similar absolute gains in sodium space and total exchangeable sodium have been reported (11, 12). Many factors have been suggested as contributing to the increased retention of

Received July 12, 1958.

* Supported by a grant from the National Research Council of Canada, the U. S. Public Health Service Grant No. H-2716, and a Federal Provincial Grant in Aid of Research Upon Anaemias in Pregnancy by the Department of Health and National Welfare of Canada.

sodium and fluid, such as alterations in renal function, changes in capillary permeability, increased venous pressure, and increased production of antidiuretic and adrenocortical hormones. The recent finding of a significant increase in the excretion of aldosterone (13–18), an active sodium-retaining hormone, in normal pregnancy, has further emphasized the participation of the adrenal cortex in these metabolic alterations.

In order to study this relationship more closely we have carried out metabolic studies in 5 normal pregnant women maintained on a constant daily intake of fluid, electrolytes, protein and calories. Studies were begun two to three weeks prior to delivery and were continued for six to twelve days in the early puerperium. Sodium, potassium and nitrogen balances were measured. Blood studies included periodic determinations of blood and plasma volume, total body fluid and extracellular fluid space. Hemoglobin concentration, packed cell volume, red cell counts, and the serum levels of sodium, potassium and nonprotein nitrogen were followed. The urinary excretion of 17-hydroxycorticosteroids and aldosterone was also determined.

CLINICAL MATERIAL

The 5 pregnant women chosen for this study were followed in the antenatal clinic; their antenatal history, and results of physical and laboratory examinations were normal throughout. The diets of all 5 patients prior to hospitalization were varied and adequate and were supplemented daily with polyvitamin and iron-containing tablets. All patients delivered normally and had no complications throughout the puerperium. They were admitted to the hospital approximately two to three weeks before the expected time of delivery and were maintained in a separate room where metabolic studies could be conducted. Three diets were prepared which were agreeable to the patients and these were rotated so that there would be some variety; however, the caloric intake as well as fluid and electrolyte content were constant for all 5 patients. The daily caloric intake varied from 2025 to 2035 calories and the fluid intake was maintained at 2000 ml. The protein intake varied from 78 to 80 Gm., and the fat and carbohydrate intakes were 83 Gm. and 224 Gm. respectively. The daily sodium intake was constant at 130 mEq., except for the days when sodium thiosulfate was administered intravenously for the measurement of extracellular fluid volume. Potassium intake was maintained at 102 mEq. A supplement of 245 mg. of vitamin C was given daily. All the food was eaten except on the day immediately before or after delivery. As it was not possible to obtain an accurate collection of the amniotic fluid, and as the blood loss at the time of delivery was only estimated, these could not be included in the balance. The results of the studies are shown in Figures 1 to 5.

The following weight changes occurred in the patients during the period of study:

Case I. J.L., a primigravida aged 25 years, weighed 130½ pounds on admission. She lost 3 pounds over a period of thirty-one days in the antepartum period. A girl weighing $6\frac{6}{10}$ pounds was delivered by forceps. The patient lost 11½ pounds at delivery and a further 5½ pounds during the 14-day postpartum period. The blood loss was estimated at 150 ml. and the amniotic fluid approximately 800 ml.

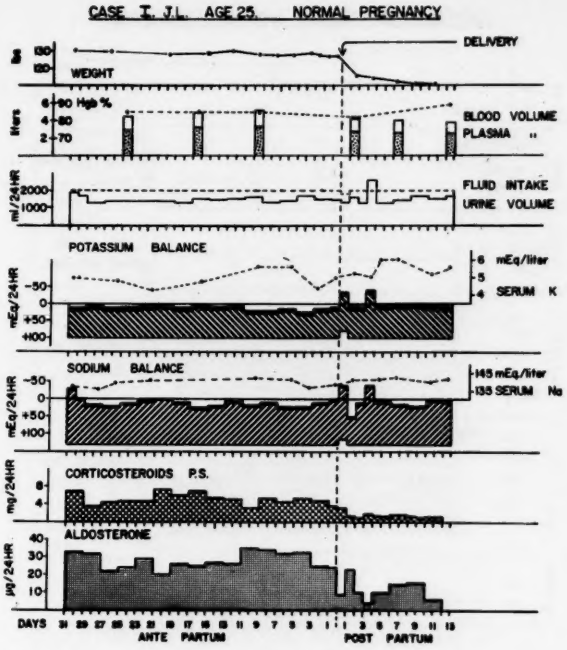

FIG. 1. Metabolic balance studies, blood volume and hormone excretion in Case I.

Case II. C.B., a primigravida aged 30 years, weighed 122 pounds on admission. She lost only $1\frac{1}{2}$ pounds during the 21-day antepartum period of study, $10\frac{1}{2}$ pounds at the time of delivery, and 8 pounds during the 9-day postpartum phase. A boy weighing $6\frac{7}{16}$ pounds was delivered spontaneously. Blood loss was estimated at 400 ml., and amniotic fluid at 450 ml.

Case III. K.B., a primigravida aged 32 years, weighed 166 pounds on admission. She lost 3 pounds during an 18-day antepartum period of study, 13 pounds at delivery, and 5 pounds during the 7-day postpartum period. A boy weighing $7\frac{9}{16}$ pounds was delivered spontaneously. Blood loss was estimated at 300 ml. There was a large volume of amniotic fluid, which was lost.

Case IV. J.B., gravida II, aged 24 years, weighed 134 pounds on admission. She lost $2\frac{3}{4}$ pounds during the first three days of the controlled diet and only $1\frac{3}{4}$ pounds during the subsequent twenty days of study in the antepartum phase. Weight loss at the time of delivery was 13 pounds. A boy weighing $8\frac{1}{3}$ pounds was delivered spontaneously. A further loss of 5 pounds occurred in the 6-day postpartum period. Blood loss was estimated at 300 ml. The amount of amniotic fluid was not measured.

Case V. E.F., a primigravida aged 30 years, weighed 160 pounds on admission. She lost $5\frac{1}{2}$ pounds during the 15-day antepartum period of study. Loss at the time of delivery was $7\frac{1}{2}$ pounds. A girl weighing $6\frac{1}{2}$ pounds was delivered spontaneously. A further weight

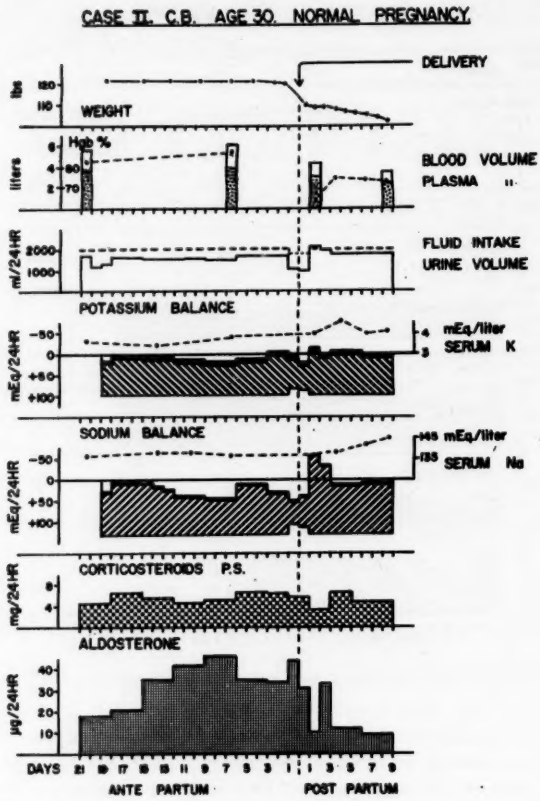

FIG. 2. Metabolic balance studies, blood volume and hormone excretion in Case II.

loss of 6½ pounds occurred during the 9-day postpartum period. Blood loss was estimated at 400 ml., and amniotic fluid at 200 ml.

METHODS

Food was purchased in large lots to cover all the metabolic studies. The dietary intake was calculated with the aid of standard food tables (19) and was frequently checked by analysis. Any uneaten food or vomitus was examined for the content of nitrogen, sodium and potassium. (Vomiting and inability to eat the food occurred only at the time of delivery.) Complete 24-hour urine collections were obtained, the accuracy of which was checked by creatinine determinations. The feces were not collected, but average values of 1.3 Gm. of nitrogen, 4.0 mEq. of sodium and 10.0 mEq. of potassium were used in calculating the balances. It has been shown that the fecal loss of nitrogen, sodium and potassium is relatively constant and is independent of the intake (20). These values were derived from results of metabolic balance studies on nonpregnant and pregnant subjects, and represent the average fecal excretion for a large number of cases. Urinary nitrogen was determined by the method of Peters and Van Slyke (21) and serum nonprotein ni-

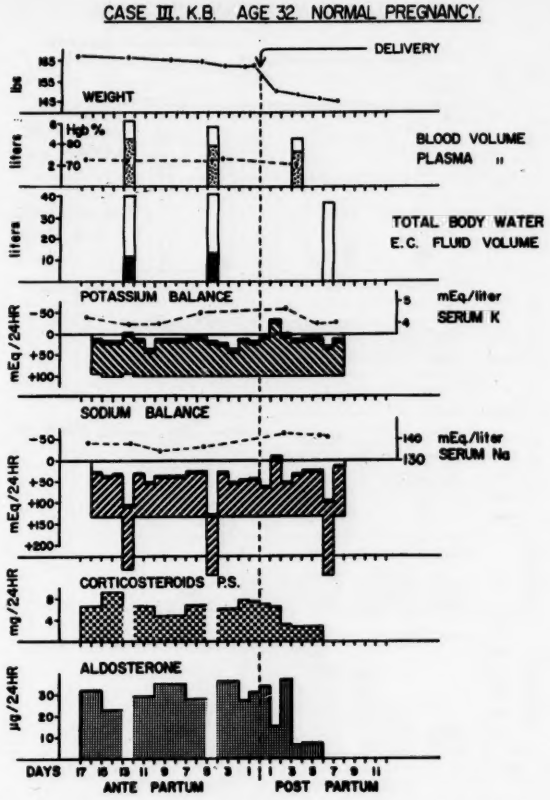

FIG. 3. Metabolic balance studies, blood volume, total body water and hormone excretion in Case III.

trogen by the method of Folin and Wu (22). Serum and urinary sodium and potassium were measured by flame photometry. Total urinary 17-hydroxycorticosteroids were determined by the method of Porter and Silber (23). The urinary aldosterone fraction was purified by means of paper chromatographic procedures in 2 solvent systems; first in the Zaffaroni (24) system and secondly in the Bush C (25) system. The purified fraction was measured by the bioassay of Venning *et al.* (26). In the antepartum period aldosterone was measured on pooled urine specimens representing 48-hour or 72-hour collections; however, near delivery and for several days afterwards, this hormone was assayed daily.

Total body water was determined by the method of Brodie *et al.* (27). A dose of 5 ml. of a 20 per cent solution of antipyrine, mixed in 30 to 40 cc. of 5 per cent glucose in water, was injected intravenously. At least 3 blood samples were obtained from a separate vein without the use of a tourniquet. The first sample was taken at three hours and the final sample at seven hours after the injection. The plasma antipyrine concentrations were determined and were plotted against time. A straight line was extrapolated to zero time. This concentration was used in the calculation of the volume of distribution.

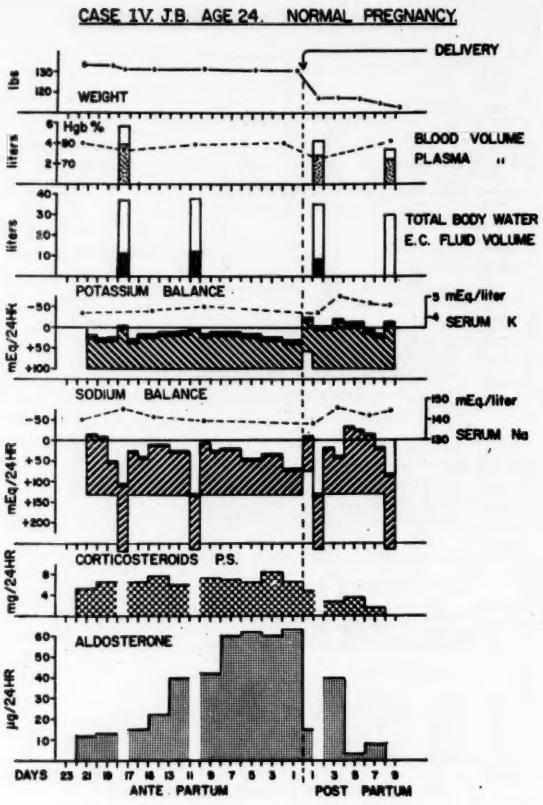

FIG. 4. Metabolic balance studies, blood volume, total body water and hormone excretion in Case IV.

The extracellular fluid space was measured by the method of Frank and Carr (28). A dose of 95 ml. of a 10 per cent solution of sodium thiosulfate was injected intravenously over a period of twelve minutes. The first blood sample was drawn at thirty minutes, and 4 more samples at eight-minute intervals.

Blood and plasma volumes were determined by the method of Fields *et al.* (29). Hematocrit readings were corrected for trapped plasma, and the total body hematocrit was calculated from the peripheral venous hematocrit reading. Red cell values were determined by methods previously detailed (4).

RESULTS

Body Weight

Although the weight of the 5 patients ranged from 120 to 166 pounds, they were maintained on the same caloric intake, which was distributed similarly with respect to protein, fat and carbohydrate. All the patients

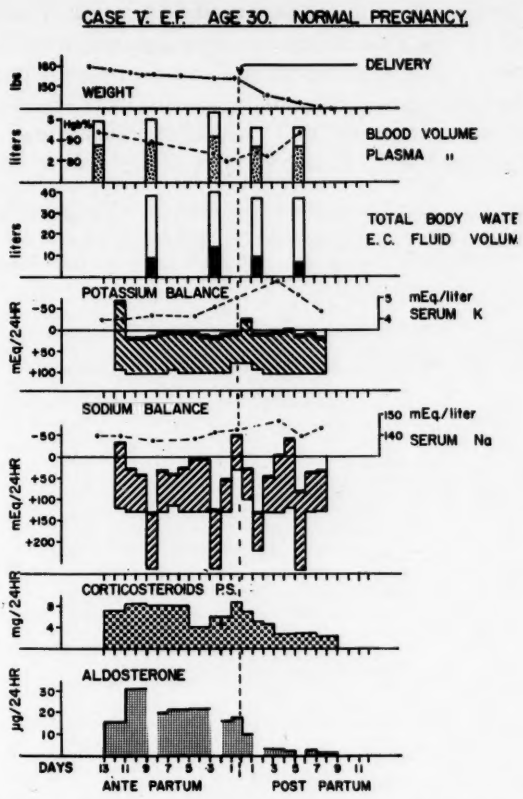

FIG. 5. Metabolic balance studies, blood volume, total body water and hormone excretion in Case V.

lost some weight ($1\frac{1}{2}$ to $5\frac{1}{2}$ pounds) during the antepartum period. This loss occurred more rapidly in Cases IV and V during the first five days of the controlled diet. At the time of delivery the weight loss, excluding the weight of the baby, varied from 1 to $5\frac{1}{4}$ pounds. In the postpartum period the rate of weight loss was accelerated. the average daily loss (grams) is shown in Table 1 for the ante- and postpartum periods. The weight loss at the time of delivery was excluded from these calculations.

Balance Studies

The balances for potassium and sodium are illustrated in Figures 1 to 5, and for nitrogen in Figure 6. The intake is plotted from the zero line downwards and the urinary excretion is plotted upwards from the intake line and is represented by the cross-hatched area. The fecal excretion is represented

by the black area at the top of the urinary excretion. Negative balances are represented by the area above the zero line, positive balances by the blank areas below this line. Although daily measurements of urinary potassium, sodium and nitrogen were made, values for forty-eight or seventy-two hours were pooled to coincide with values for aldosterone determined on similarly pooled specimens. Because rapid changes were occurring at the

TABLE 1. AVERAGE DAILY BALANCE FOR NITROGEN, POTASSIUM AND SODIUM IN ANTEPARTUM AND POSTPARTUM PERIODS; EXCRETION OF ALDOSTERONE AND 17-OH-CORTICOSTEROIDS; AND AMOUNT OF WEIGHT LOSS

	Period (days)	Weight loss (Gm./24 hrs.)	Balance			Excretion	
			N (Gm./24 hrs.)	K (mEq./24 hrs.)	Na (mEq./24 hrs.)	Aldosterone (μg./24 hrs.)	17-OH-CS (mg./24 hrs.)
Case 1	30—20 AP*	90.6	+0.7	+ 6	+13	27	5.3
	20—10 AP	0.0	+1.5	+ 7	+15	26	5.5
	10— 1 AP	68.0	+1.7	+17	+17	31	4.3
	Delivery	5220.0					
	1—12 PP	181.0	−0.2	+ 4	+ 6	12	3.1
Case 2	19—10 AP	0.0	+0.9	+ 4	+18	26	5.6
	10— 1 AP	90.6	+1.6	+11	+34	45	5.6
	Delivery	4760.0					
	1— 9 PP	362.0	−0.9	− 2	+ 6	19	4.9
Case 3	15—10 AP	45.3	+1.4	+13	+36	28	7.0
	10— 1 AP	90.6	+1.3	+13	+37	32	5.5
	Delivery	5900.0					
	1— 6 PP	159.0	−0.3	+ 1	+21	17	3.9
Case 4	20—10 AP	68.0	−0.5	+12	+23	20	6.3
	10— 1 AP	45.3	+1.4	+17	+41	55	6.7
	Delivery	5900.0					
	1— 8 PP	226.0	−0.8	− 7	0	16	3.0
Case 5	10— 1 AP	113.3	+0.9	+ 6	+18	19	6.8
	Delivery	3600.0					
	1— 6 PP	125.0	−0.8	− 2	+ 8	5	3.8

* AP = ante partum.
PP = post partum.

time of delivery and in the early puerperium, measurements of hormone excretion and balances were made daily.

The balances in the postpartum period did not include losses from discharge of blood and lochia or loss through the skin. According to the recent metabolic study of de Alvarez *et al.* (30) this may involve significant amounts of nitrogen, sodium and potassium during the first three days after delivery. The patients in our study did not lactate. In order to correlate more readily the changes in hormone excretion and electrolyte balance with the changes in fluid volume spaces, values for periods of ten days or less were averaged and are shown in Table 1. Results are expressed as an average daily amount for each period.

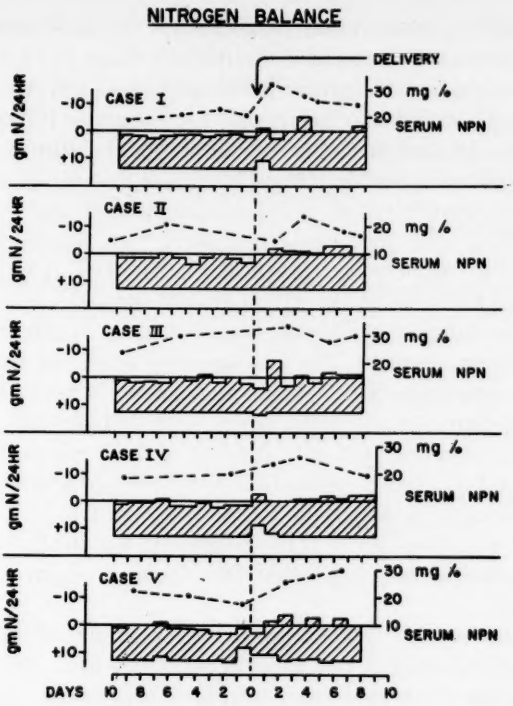

FIG. 6. Nitrogen balance studies in Cases I, II, III, IV and V. (Serum nonprotein nitrogen concentration, mg. per 100 ml.; nitrogen excretion, Gm. per 24 hours.)

Nitrogen balance

The length of the balance studies in the 5 cases varied in the antepartum period from two to four weeks. In the ten-day period before delivery all patients showed a positive nitrogen balance varying from an average of 0.9 to 1.7 Gm. of nitrogen per day (Table 1). The daily variations in balance for the periods just before and after delivery are shown in Figure 6. In the earlier periods similar amounts of nitrogen were retained except in Case IV, in which a slightly negative nitrogen balance of 0.5 Gm. per day was observed between the twentieth and tenth days. All 5 patients showed an overall negative nitrogen balance in the postpartum period, ranging from an average of −0.2 Gm. to −0.9 Gm. per day. Following delivery the initial rise in urinary nitrogen was associated with a simultaneous diuresis of fluid, potassium and sodium. It was impossible to account for the loss of nitrogen due to bleeding and discharge of lochia during the postpartum period; therefore, negative nitrogen balances at this time are probably greater than the values indicated.

In our laboratory, the normal range for the level of serum nonprotein nitrogen (NPN) in nonpregnant individuals is from 15 to 30 mg. per 100 ml. In the cases studied, the serum NPN level remained within the normal range except in Case III, in which it rose to 34 mg. per 100 ml. on the third postpartum day. In all 5 cases the concentration of serum NPN was higher post partum than ante partum (Fig. 6).

Potassium balance

The potassium balances are shown in Figures 1–5. A value of 10 mEq. was used for the fecal excretion. All 5 patients showed a retention of potassium during the antepartum period, and in 3 of them this increased as the time of delivery approached. The average daily retention for periods of ten days or less is shown in Table 1; it ranged from 4 to 17 mEq. per day. Within twenty-four to forty-eight hours after delivery there was a rise in urinary potassium, associated with diuresis of fluid and sodium. In Cases I and IV, a second diuresis of potassium was observed a few days later. The overall balance in the postpartum period was negative in 3 cases and only slightly positive in the other 2. If the potassium lost in the blood and lochia had been included, all patients would have shown a negative balance in this period.

In this laboratory, the normal range for serum potassium concentration in the nonpregnant subject is 4.1 to 5.5 mEq. per liter. Before delivery, in the present series, the serum potassium level was within the normal range in 4 patients (Figs. 1, 3, 4 and 5); only in Case II were low values of 3.1 and 3.6 mEq. observed (Fig. 3). The serum potassium concentration rose in all patients following parturition, and in Case V reached a level of 6.0 mEq. per liter on the fifth day post partum.

Sodium balance

The dietary intake of sodium was maintained at 130 mEq. per day in all 5 patients. In Cases III, IV and V an additional load of approximately 130 mEq. of sodium in the form of thiosulfate was given intravenously on two occasions during the antepartum period and again in the postpartum period, for the determination of extracellular fluid volume. The amount of sodium excreted on the day of the tests, expressed as a percentage of total intake, is shown in Table 2. Before delivery this ranged from 40.2 to 58.5 per cent of the total intake for that day. When this test was repeated on the second day post partum, little change was observed from values obtained before delivery. When sodium thiosulfate was administered later, on the sixth, seventh and ninth days, 65 to 68 per cent of the sodium was excreted, showing a definite increase over the amount eliminated in the antepartum period. The average daily sodium retention for the various

antepartum periods varied from 13 to 41 mEq. per day (Table 1). The balances for the days on which the sodium load was given were not included in these calculations. In Cases II and IV a progressive increase in sodium retention was observed prior to delivery. After delivery, diuresis occurred within twenty-four to forty-eight hours. In Cases I, IV and V this was followed by retention and a second diuresis. In Case V (Fig. 5) a negative sodium balance was observed on the day prior to delivery. As the baby was born at midnight and the urines were collected from 8:00 A.M. to 8:00

TABLE 2. PERCENTAGE OF SODIUM EXCRETED BEFORE AND AFTER DELIVERY, FOLLOWING ADMINISTRATION OF SODIUM THIOSULFATE

Day	Case 3	Case 4	Case 5
Ante partum			
18	—	58.5	—
13	56.5	—	—
11	—	49.0	—
9	—	—	49.5
5	52.0	—	—
3	—	—	40.2
Post partum			
2	—	49.0	39.4
6	—	—	68.0
7	65.0	—	—
9	—	68.0	—

A.M., some of the urine obtained post partum was included in the specimen for this day. The sodium balance in this patient was obscured by the administration of sodium thiosulfate close to the time of delivery and again on the second postpartum day. Because of the interference of this substance with the extraction of aldosterone, no assay of this hormone could be carried out on these days. The overall sodium balance remained positive during the postpartum period in all patients, although considerably less sodium was retained at this time than prior to delivery. If loss of sodium from other sources, such as blood, lochia and sweat had been included, the positive balance would have been reduced further.

In our laboratory, the normal range for serum sodium concentration in the nonpregnant subject is 139 to 148 mEq. per liter. In Cases II and III the serum sodium levels remained below this range prior to delivery. In Cases I and V they varied between 137 to 141 mEq. per liter and in Case IV between 140 and 145 mEq. per liter. Following delivery the levels tended to increase. Only in Case II was a progressive rise in serum sodium concentration observed in the postpartum period.

Urinary Corticosteroids

Aldosterone

Urinary aldosterone excretion showed a wide range of values in the 5 patients during the antepartum period, but all values were higher than those observed in nonpregnant women. In Cases I and III (Figs. 1 and 3) the excretion remained fairly constant and averaged 28 to 30 μg. per day, whereas in Cases II and IV (Figs. 3 and 4) there was a progressive rise in excretion up to the time of delivery. In Case II the rise was from 18 μg. to 47 μg., and in Case IV from 13 μg. to 63 μg. per twenty-four hours. Within twenty-four to forty-eight hours after delivery, there was a sharp fall in the excretion of aldosterone which was usually followed by a temporary rise which lasted for one or two days. Then excretion decreased abruptly to values found in nonpregnant subjects. In Case I the levels increased for a second time between the sixth and tenth postpartum days. This was not observed in the other 4 cases. The administration of sodium thiosulfate for the determination of extracellular fluid space appeared to interfere with the quantitative extraction of aldosterone from the urine and no data were obtained on the days that these tests were performed. The average values for aldosterone excretion in the various periods are listed in Table 1. In the 2 patients (Cases II and IV) who showed increased retention of sodium in the ten-day period prior to delivery, the average values for aldosterone excretion were also higher during this period.

17-Hydroxycorticosteroids

Although fluctuations were observed in the excretion of 17-OH-corticosteroids (Porter-Silber), the values were higher in the antepartum period than in the postpartum period. Before delivery the average values ranged from 4.3 to 7.0 mg., and after delivery, from 3.0 to 4.9 mg. per twenty-four hours. The normal range for nonpregnant females is from 2.0 to 5.5 mg. per twenty-four hours. In 4 of the patients there was a decrease to nonpregnant levels within three days after delivery. In Case II only a slight fall in excretion occurred following parturition. The wide variations observed in the daily excretion of aldosterone in the postpartum period were not noted in the excretion pattern of 17-hydroxycorticosteroids.

Total Body Water and Extracellular Fluid Volume

The total body water and extracellular fluid volumes were determined by Dr. L. E. McLeod (31). They were measured on two occasions before delivery and again after delivery. Of the many methods developed for determining total body water, the antipyrine method has been widely used in nonpregnant subjects. Only a few studies, however, have been

carried out in pregnant women. Lambiotte & Escoffier *et al.* (32) have shown that antipyrine readily passes through the placenta and is equally distributed in maternal plasma and amniotic fluid. In the present studies the disappearance of antipyrine from the plasma was adequately linear. The penetration of sodium thiosulfate into the amniotic sac was unknown. The rate of disappearance of sodium thiosulfate from the plasma (used for determination of extracellular fluid volume) was occasionally irregular. These values were discarded, but it is possible that variations in distribu-

TABLE 3. CHANGES IN BODY WATER IN PREGNANCY, BEFORE AND AFTER DELIVERY

	Day	Weight (Kg.)	Volume of distribution		
			Antipyrine		Sodium thiosulfate (liters)
			(liters)	(% of wt.)	
Case 3	Ante partum				
	13	75.2	39.8	53	11.1
	5	74.3	41.3	56	12.9
	Post partum				
	7	65.6	37.0	56	—
Case 4	Ante partum				
	18	59.6	37.1	62	10.9
	11	59.4	38.6	64	11.9
	Post partum				
	2	53.2	35.0	66	8.6
	9	51.0	30.7	60	—
Case 5	Ante partum				
	9	70.5	38.3	54	9.0
	3	69.7	39.8	57	14.5
	Post partum				
	2	66.0	37.1	56	9.7
	6	64.8	37.1	57	6.8

tion may have produced an error in the thiosulfate spaces reported here. The values obtained for total body water and extracellular fluid volume are listed in Table 3. Both the antipyrine space and the thiosulfate space increased during the last week of pregnancy in the presence of some loss of weight. Following delivery there was a contraction in both spaces.

Blood and Plasma Volume

The total blood, plasma and red cell volumes antepartum were increased above those in nonpregnant normal subjects in every instance and were

within the range which has been observed during the last calendar month of normal pregnancy (4).

Before delivery, as shown in Table 4, serially determined blood and plasma volumes increased in Cases I and V, decreased in Case III, and showed no significant change in Case II. The determination was performed only once in Case IV. It was not possible to correlate these changes closely with urinary 17-hydroxycorticosteroid or aldosterone excretion, although the latter was elevated at the time of these measurements. Consistent with previously reported observations (4, 33) the blood loss was much greater when calculated from the difference between the last antepartum and first postpartum blood volumes. Thus the estimated blood loss in Cases II and V was 400 ml. each, and in Cases III and IV, 300 ml. each. By blood volume determinations the blood loss was 2173 ml. in Case II, 1168 ml. in Case V, 2108 ml. in Case III, and 1050 ml. in Case IV.

Postpartum blood volume determinations were too few in number and not spaced closely enough for accurate correlation with the changes in 17-hydroxycorticosteroid and aldosterone excretion. From the data presented, there seemed to be a tendency for the plasma volume and urinary aldosterone excretion to return to nonpregnant normal levels at about the same time. It is unfortunate that the nature of the technique prevented daily blood volume determinations.

In Case V the progressive rise of plasma volume before delivery accounted largely for the drop of 10 per cent in hemoglobin concentration and the decrease of packed cell volume (P.C.V). In Case I, both the total red cell volume and plasma volume increased slightly and consequently there was no significant change in the hemoglobin concentration or P.C.V. After delivery, in Cases I, IV and V, although the total red cell volume decreased significantly, the concomitant decrease in plasma volume prevented significant changes of hemoglobin concentration or P.C.V. Blood loss as measured by changes in blood volume was greatest in Case II, and in this patient the hemoglobin concentration and P.C.V. dropped significantly post partum. Although the estimated blood loss was 400 ml., the findings suggest that this patient may have suffered from a significant degree of postpartum hemorrhage. Individual variations of blood volume and hematologic values similar to those shown by these patients, both ante partum and post partum, have been reported previously for normal pregnancy (29).

There would not appear to be any correlation between changes in plasma volume and in sodium thiosulphate space. In Case V the sodium thiosulfate space increased from 9.0 to 14.5 liters from the ninth to the third antepartum days while the plasma volume increased from 3653 to 4270 ml. In Case III the sodium thiosulfate space increased from 11.1 to 12.9 liters be-

TABLE 4. BLOOD VOLUME DATA

	Day	Blood vol. (ml.)	Plasma vol. (ml.)	Red blood cell vol. (ml.)	Hgb. (%)	Packed cell vol. (%)
Case 1	Ante partum					
	36	4633	3002	1590	87	39
	25	4528	2981	1515	85	38
	18	4991	3314	1633	85	37
	10	5215	3447	1731	85	38
	Post partum					
	2	4272	2813	1399	82	37
	7	4137	2743	1338	89	37
	10	3846	2450	1361	90	40
Case 2	Ante partum					
	14	5888	3898	1948	84	37
	7	6107	4011	2042	88	38
	Post partum					
	2	4531	3255	1216	64	30
	9	3549	2506	1003	73	32
Case 3	Ante partum					
	13	6233	4437	1769	72	32
	5	5753	3826	2056	72	37
	Post partum					
	4	4537	3212	1296	70	32
	7	4307	3063	1179	—	31
Case 4	Ante partum					
	18	5688	3929	1708	77	34
	11	—	—	—	80	39
	Post partum					
	2	4217	2855	1351	74	36
	9	3401	2305	1081	82	36
Case 5	Ante partum					
	15	5804	3468	2277	94	45
	9	5883	3653	2348	89	32
	3	6479	4270	2175	84	38
	Post partum					
	2	5077	3288	1731	84	40
	6	5119	3313	2052	94	43

tween the thirteenth and fifth antepartum days while in the same period the plasma volume decreased from 4437 to 3826 ml.

DISCUSSION

Although the adrenal cortex has repeatedly been implicated in the increased retention of fluid and sodium during pregnancy, this is the first attempt to correlate excretion of corticosteroids and aldosterone with measurements of fluid spaces and metabolic balance studies. Our findings of a continued retention of nitrogen and electrolytes in normal pregnant women prior to delivery, with diuresis following parturition, agrees with the reports of previous investigators (34–39). It is difficult to compare the quantitative aspects of these earlier results with our findings because of the wide range in intake, the varied reliability of methods used for determining sodium and potassium, and the failure in some instances to consider fecal excretion. The present study shows the variability of these parameters of adrenal function in 5 normal pregnant women maintained with the same fluid and electrolyte intake over periods of two to four weeks before parturition. The balance patterns differed from case to case and bore no relationship to the weight of the patient. In 2 instances there was a rapid accumulation of sodium and potassium in the last week of pregnancy. When average daily retentions of sodium and potassium were considered for ten-day periods in the antepartum phase, the gain in sodium varied from 13 to 41 mEq. and that of potassium from 4 to 17 mEq. per day. In each patient, however, the excretion of sodium and potassium followed somewhat similar trends, although the magnitude of the changes in sodium balance were greater than those in potassium balance.

The excretion of nitrogen, potassium and sodium fluctuated considerably post partum, although the patients continued to be maintained on the same intakes. There appeared to be definite periods of diuresis of these various constituents, which occurred simultaneously—the first within twenty-four to forty-eight hours after delivery and in some cases a second diuresis a few days later. This resulted in an overall negative nitrogen balance, and a potassium balance that tended to come into equilibrium or to be slightly negative. The overall sodium balance for the short period of study in the puerperium remained positive, but was greatly reduced compared with that observed before delivery. If accurate allowances could be made for the additional losses due to various discharges that were not measured, these balances would be further reduced.

Conflicting findings have been reported with regard to the concentration of sodium and potassium in the serum of pregnant women. Only in 1 patient was the serum potassium level below our normal range for the nonpregnant state; however, following delivery, there was a tendency toward

higher concentrations of this electrolyte in all cases. Variations were also observed in the concentration of serum sodium. In 2 of the patients the sodium levels were consistently below our normal range for nonpregnant subjects in the antepartum period; in the other 3 patients the values remained within the normal range. These variations in serum sodium concentration appeared to be independent of changes in sodium balance. In all the patients the levels of serum sodium were higher in the postpartum period, in agreement with recent studies reported by Goodland *et al.* (40).

It is of interest that although there was a gain of nitrogen, potassium and sodium in the antepartum period, the patients gradually lost weight. The most likely explanation is an insufficient caloric intake. Weight loss was most marked in the largest patient in the group and least marked in the smallest, an observation which supports this concept. The weight loss could not be explained by loss of water due to periodic measurements of total body fluid, extracellular fluid and plasma volume. In fact, there appeared to be a slight expansion of the various fluid spaces near term in some of the patients. The positive nitrogen and potassium balance and the increase in extracellular fluid volume in the face of gradual weight loss strongly suggest mobilization of fat from adipose tissue depots to supply the energy requirements for fetal protein synthesis. Comparable metabolic data were obtained on obese subjects during a period of weight loss accomplished by caloric restriction but adequate protein intake. It is tempting to ascribe the foregoing observations to increased secretion of growth hormone. Following parturition, all the fluid volume spaces decreased; the patients were not followed long enough, however, to allow for complete metabolic readjustments to occur after delivery, and stable conditions had not been reached before termination of the study. The values for total body water are in accord with amounts previously reported by other workers in this field and agree with results obtained by Seitchik and Alper (9) using the antipyrine method. These investigators observed a gradual rise in total body water in 5 normal pregnant women and in each pregnancy the total body water gained was greater than the weight gained. Lambiotte-Escoffier *et al.* (32), using the same method, concluded that there was no striking change in the antipyrine space in normal pregnancy but these conclusions appeared to be based upon single determinations in 2 pregnant women in the antepartum period only. When the total body water was expressed as a percentage of the weight, no consistent trend was observed. According to Pace and Rathbun (41), this manner of expression represents an estimate of the relative obesity of the patient. Seitchik and Alper (9) showed that changes in body composition occur in normal pregnancy and suggested that utilization of stored fat is required for the synthesis of lean tissue. The anabolic state in pregnancy is characterized by a gain in protoplasmic

mass and loss of fat. This process is reversed in the puerperium. These authors suggest that the proportionate increase in all body compartments is a normal and integral part of the accumulation of lean body mass by mother and fetus. The methods available for the determination of extracellular fluid volume are all subject to criticism. Although the distribution of sodium thiosulfate appears to be satisfactory in the nonpregnant subject, it occasionally shows irregularities in pregnant women, and its distribution in the amniotic sac is unknown.

It has frequently been suggested that the continued retention of sodium and expansion of body fluids in normal pregnancy is a result of the higher levels of adrenocortical hormones in the plasma and urine of pregnant women. This theory has been given further support by the isolation of a potent sodium-retaining hormone from adrenal extracts by Simpson *et al.* (42) and the demonstration by Venning and Dyrenfurth (13) of a progressive rise in the excretion of this hormone in pregnancy. This increased level of urinary aldosterone in late pregnancy has been confirmed by various investigators (15–18). Although the present studies show that wide variations can occur in the excretion of aldosterone near term, the values obtained in the last trimester were usually ten to twenty times those observed in the nonpregnant woman. In 2 of the pregnant women an increased retention of sodium in the last ten days of pregnancy was associated with a higher average output of aldosterone (Table 1). In another pregnant woman in whom the sodium retention remained fairly constant over a similar period, the excretion of aldosterone varied only slightly. With the exception of 1 case, rapid changes occurring in sodium excretion in the early postpartum period were inversely related to the levels of aldosterone, the early diuresis of sodium being associated with a decrease in aldosterone excretion, and the subsequent retention of sodium, with an increase in aldosterone excretion. Between the fourth and fifth days of the puerperium, urinary aldosterone decreased to values usually obtained in nonpregnant women. In contrast to findings observed in the antepartum and immediate postpartum periods, no further changes occurred, although sodium excretion was varying at this time. It is difficult to evaluate the effect of aldosterone on potassium metabolism in pregnancy. In nonpregnant subjects, administration of aldosterone leads to sodium retention and increased potassium excretion. In our studies, there was an increase in potassium retention at a time when aldosterone excretion was high and sodium was also being retained—a phenomenon probably associated with fetal protein storage. The stimulus for the increased excretion of aldosterone in pregnancy is unclear at the present time. In normal subjects, secretion of this hormone may be stimulated by a variety of conditions. Reduction in dietary intake of sodium (43) affects aldosterone excretion.

Contraction of total body or extracellular fluid leads to a rise in the output of aldosterone, and expansion of extracellular fluid space or blood volume results in a fall in the levels of this hormone (44–47). The latter effects are independent of changes in the sodium concentration of extracellular fluid and suggest that aldosterone may be controlled by some function of fluid volume. In pregnancy, the excretion of aldosterone rises in the presence of an expanded total fluid and blood volume. A similar situation is observed in some patients with cirrhosis and cardiac failure when edema is present. The more recent observations of Bartter (48) indicate that control of aldosterone secretion may depend upon some function of the intravascular volume. Our present studies on the measurement of various fluid spaces in pregnancy do not contribute much to our understanding of this problem. It is possible that the demands of the rapidly growing uterus and products of conception may in some manner alter the fluid balance in the maternal organism and lead to a decrease in the still undefined "effective intravascular volume" controlling aldosterone secretion. This would result in an increase in aldosterone secretion with a consequent retention of sodium, and restoration of the "effective intravascular volume." At present, satisfactory methods are not yet available to test this theory.

Other hormonal factors, such as estrogens and 17-hydroxycorticosteroids may also contribute to changes in sodium balance during pregnancy. Earlier studies of Taylor *et al.* (37) indicated that rising levels of estrogens in pregnancy may be associated with sodium retention. Administration of this hormone prevented the diuresis of sodium normally observed after parturition. The increased levels of blood corticosteroids and the higher excretion of these hormones in pregnancy have also been thought to account for the continued sodium retention. The fluctuations in sodium balance following parturition did not appear to be related to the excretion of these hormones. The excretion of both 17-hydroxycorticosteroids and estrogen decreased steadily following parturition.

It has been suggested that the placenta may elaborate adrenocortical hormones, and aldosterone has been extracted in small amounts from this tissue (49). The present studies would favor the maternal adrenal gland rather than the placenta as the source of this hormone, as aldosterone continues to be excreted in large amounts following the separation of the placenta. Newborn infants excrete only small amounts of aldosterone (1–2 μg. per day), indicating the fetal adrenal secretion does not influence to any great extent the total output of aldosterone in pregnancy. This concept is supported by the findings of Laidlaw *et al.* (50), who reported very low excretions of aldosterone (1.0 μg. and 4.4 μg.) in 2 adrenalectomized pregnant women. Our findings in 2 similar patients (unpublished results)

indicated that one of them did not excrete any detectable amount of aldosterone in the urine, whereas the other excreted 10 μg. daily. Following delivery, aldosterone excretion decreased to 2.5 μg. in the second case. Beaulieu *et al.* (51) were unable to detect cortisol in the plasma of a pregnant woman with Addison's disease.

Acknowledgments

Grateful acknowledgment is made to Dr. M. Sabin who coordinated the clinical care and investigation of the patients; to Dr. Laura Caligaris who carried out many of the aldosterone assays; and to Miss Madelein Lalonde and Miss Laura Gingras for their technical assistance.

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SERUM GLUTAMIC-OXALACETIC TRANSAMINASE (SGO-T) AND SERUM GLUTAMIC-PYRUVIC TRANSAMINASE (SGP-T) IN TOXEMIA OF PREGNANCY

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ABSTRACT

Determinations were made of the serum glutamic-oxalacetic transaminase and serum glutamic-pyruvic transaminase levels in patients with toxemia of pregnancy and in pregnant women with symptoms of liver damage supposed to be secondary to the pregnant state. The transaminase value was found to be high in about 30 per cent of the former and in more than 50 per cent of the latter. The only demonstrable relationship between a high transaminase value and clinical symptoms or other laboratory findings was the correlation with signs of co-existing liver disorder or damage. The assumption that the increase in transaminase activity is due to hepatocelluar damage is also supported by the observation that the increase in the serum glutamic-oxalacetic transaminase value was usually accompanied by an increase in the serum glutamic-pyruvic transaminase value, and that in some cases only the latter was increased above the normal level.

NEITHER normal pregnancy nor many of the complications of pregnancy have any significant influence on the activity of serum glutamic-oxalacetic transaminase (SGO-T). Only in cases of toxemia of pregnancy have high SGO-T values been reported (1–3). Such an increase may be expected in about 1 out of every 3 cases of toxemia of pregnancy (2). The increased activity is not correlated with the severity of toxemia, the SGO-T values sometimes being very high in cases of toxemia of pregnancy with slight to moderate symptoms. The SGO-T increase is believed to be due to liver damage (2), but convincing evidence for such an assumption is still lacking.

In an attempt to elucidate this problem it was considered worth while to determine the serum level not only of SGO-T, but also of glutamic-pyruvic transaminase (SGP-T) in a fairly large series of women with toxemia of pregnancy. An increase in SGP-T activity is probably a more specific sign of liver damage (4, 5). The present material included women with toxemia of pregnancy as well as pregnant women without symptoms of

Received September 5, 1958.

toxemia, but with symptoms of liver damage supposed to be due to pregnancy (hepatosis (6)). The serum transaminase activity was compared with the severity of the toxemia, with symptoms of renal or liver damage, and with other symptoms that might be capable of enhancing the serum transaminase value.

CLINICAL MATERIAL AND METHODS

The subjects were 53 pregnant women with symptoms of toxemia or of liver damage. Toxemia of pregnancy was said to be present if proteinuria, increased blood pressure or pronounced edema could be demonstrated during the last trimester of pregnancy and could not be ascribed to other diseases such as chronic nephropathy. In Sweden severe toxemia of pregnancy has become rare because practically all expectant mothers attend the prenatal clinic from early pregnancy (7). This explains why the series included only 1 case that might be classified as eclampsia. A survey of the data is given in Table 1.

TABLE 1

Patients with toxemia of pregnancy	No. of patients	Laboratory findings		
		Signs of hepatic damage	Increased SGO-T and/or SGP-T	Increased SGO-T and/or SGP-T+signs of hepatic damage
Group 1: mild symptoms	18	6	5	3
Group 2: moderate symptoms	20	5	6	4
Group 3: severe symptoms	8	4	2	2
	46	*15*	*13*	*9*
Group 4: "hepatosis"	7	4	4	3
Total number	*53*	*19*	*17*	*12*

In 7 of the 53 cases the picture was dominated by symptoms of liver damage, *i.e.*, high serum bilirubin value, abnormal thymol turbidity, or other signs supposed to be due to impaired liver function or liver damage (6).

All of the patients were hospitalized. The blood pressure was measured once or twice daily. The urine output and the degree of proteinuria were determined every day. As a rule, weekly determinations were made of the bile pigments in the urine, the bilirubin content and the thymol turbidity of the serum, the serum levels of nonprotein nitrogen, prothrombin, total protein, SGO-T and SGP-T. The ocular fundi were examined at varying intervals. In some cases determinations were made also of the serum levels of aldolase, alkaline phosphatase, zinc sulphate turbidity, iron, copper, phospholipids and cholesterol. Occasionally, the bromsulphalein test was performed.

SGO-T and SGP-T activities were determined by the method of Karmen *et al.* (8), as modified by Biörck and Hanson (9) for SGO-T. The enzyme activity was expressed

as units per milliliter of serum. One unit is the amount of transaminase that will decrease the optical density at $\lambda = 340$ mμ by 0.001 in one minute.

The normal values found by these methods for healthy nonpregnant women in the child-bearing age are: SGO-T, 17.9 ± 2.70 units s.d. (n = 22, range 13.7–23.0 units); SGP-T, 9.8 ± 2.36 units s.d. (n = 24, range 6.7–17.0 units).

In healthy pregnant women in the eighth to ninth month of gestation the normal values are: SGO-T, 21.8 ± 8.53 units s.d. (n = 21, range 11.5–44.4 units); SGP-T, 13.5 ± 5.48 units s.d. (n = 21, range 5.9–25.6 units).

RESULTS

The patients were divided into 4 groups. The first 3 groups included 46 patients with toxemia of pregnancy of varying severity. The fourth group consisted of the remaining 7 women in whom the clinical picture was dominated by symptoms of liver damage, the symptoms of toxemia being absent or very slight. The groups are listed in Table 2.

TABLE 2

	No. of patients	Highest transaminase value recorded in each patient			
		SGO-T		SGP-T	
		Mean	Range	Mean	Range
Toxemia of pregnancy:					
Group 1	18	35.3	11.9–161	28.6	6.3–146
Group 2	20	98.1	14.4–926	78.3	7.0–690
Group 3	8	49.2	18.5–167	28.8	11.9– 89
"Hepatosis":					
Group 4	7	64.4	17.0–184	49.9	9.7–126

Group 1 consisted of 18 patients with negligible or mild symptoms, namely slight proteinuria (at most, 1 Gm. per 1000 ml.), a blood pressure no higher than 130/80 mm. Hg, slight edema at most, and no other symptoms of toxemia of pregnancy. As judged by the usual criteria (10), these patients had no toxemia of pregnancy.

In 5 (28 per cent) of this group the SGO-T or SGP-T activity was high. In 3 of the 5, both SGO-T and SGP-T values were high; in the other 2, only the SGP-T. In 6 cases the serum bilirubin level was high and/or thymol turbidity was abnormal. In 2 cases, however, the serum bilirubin value was near the upper limit of normal (0.9 mg. per 100 ml.). In 4 patients no liver function tests were performed. In 3 of those cases in which the levels of SGO-T and/or SGP-T were increased, signs of impairment of liver function were also found. In 1 patient liver function tests revealed no signs of a pathologic condition and in 1 no such tests were performed.

Group 2 consisted of 20 patients. In all of them the symptoms were more pronounced than in the first group, namely, proteinuria (at most 2 Gm. per 1000 ml.), blood pressure no higher than 160/100 mm. Hg, moderate edema, but no other symptoms of toxemia of pregnancy. In Groups 1 and 2 the clinical condition thus corresponded roughly to "mild pre-eclampsia" according to Dieckmann's classification (10).

In Group 2 there were 6 patients in whom the activity of SGO-T as well as of SGP-T was increased. Five of the 6 showed signs of impaired liver

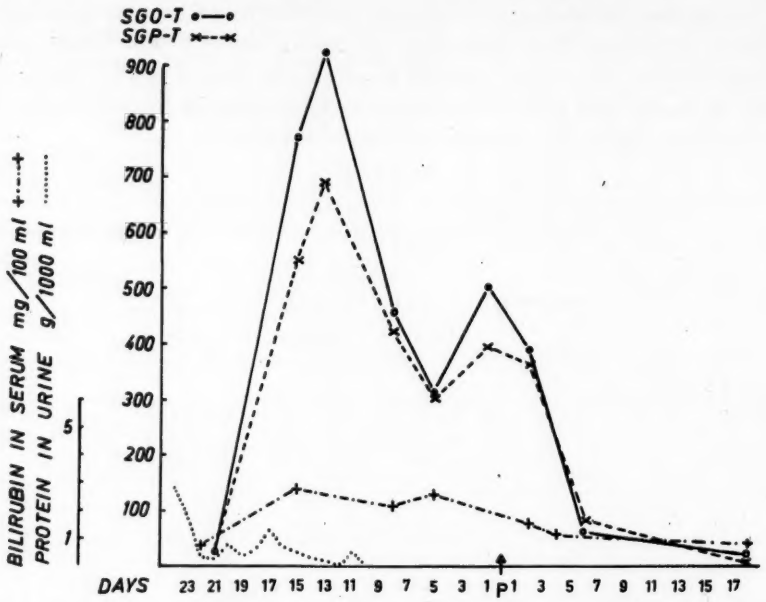

FIG. 1. Patient S.A. (412/58), *Group 2*. No increase in blood pressure, moderate proteinuria. Bromsulphalein test: 35 per cent retention. Mild pre-eclampsia with signs of liver damage and very high serum transaminase values. Abscissa: days before and after parturition (P).

function, though in 1 it was only slight, the serum bilirubin level being at most 0.9 mg. per 100 ml. In 1 case in which the SGO-T and SGP-T values were within the normal range, the results of the thymol turbidity test were somewhat abnormal (an extinction value of 0.11). The most pronounced increases in transaminase activity were found in this group (Fig. 1).

Group 3 consisted of 8 patients. These were the more severe cases of toxemia of pregnancy, corresponding to "severe pre-eclampsia" and "eclampsia" according to Dieckmann (10). Only 1 of the 8 patients had had sei-

zures. In 2, SGO-T as well as the SGP-T activity was high, and both patients showed signs of liver damage. Two other patients with normal transaminase activity had slight liver damage (serum bilirubin level, at most, 0.9 mg. per 100 ml. and a thymol turbidity value of 0.13).

In 1 of the cases in Group 3, severe nephropathy developed in association with an increasing serum level of nonprotein nitrogen and oliguria, necessitating rapid induction of labor. Despite the renal insufficiency, the serum transaminase level was normal throughout pregnancy (Fig. 2).

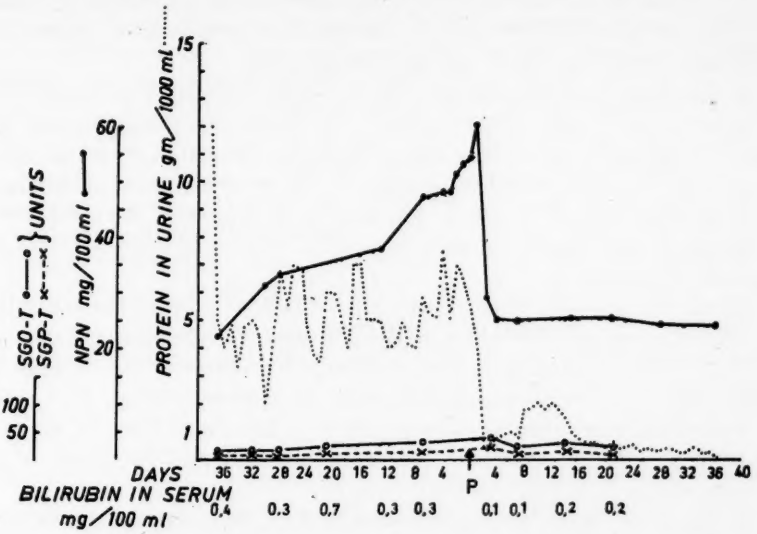

FIG. 2. Patient J.J. (727/58), *Group 3*. Severe pre-eclampsia with signs of several renal damage, increasing serum concentration of nonprotein nitrogen, and oliguria. Severe proteinuria. Moderately increased blood pressure. No signs of liver damage. Bromsulphalein retention 8 per cent. Normal serum transaminase values. Abscissa: days before and after parturition (P).

One of the patients had eclamptic convulsions before parturition. Transaminase activity was slightly enhanced, but there was also evidence of liver damage.

Group 4 consisted of 7 patients in whom there was reason to assume liver damage. Most of them had generalized pruritus. These cases were classified as hepatosis according to Arfwedson (6). Pregnancy was the only explanation that could be offered for the disorder. The patients had no symptoms of infectious hepatitis, obstructive jaundice, or hemolytic anemia. In 4 of the 7, the serum transaminase value was abnormally high.

DISCUSSION

High serum transaminase activity is believed to be a sign of cellular damage (11). However, it is only in certain diseases that the transaminase level is raised. According to Chinsky *et al.* (3), who examined a large series of patients, a high SGO-T level is most frequently a sign of hepatic or myocardial damage. Wroblewski and LaDue (4) found SGP-T activity to be raised mainly in patients with liver damage. In animal experiments it has been possible to raise the transaminase level by injuring the kidney with intraperitoneal injections of uranylacetate (12) as well as by ligating the renal vessels (11), but no such increase has been found in human subjects with renal diseases of various etiology (13).

In the present series in which the cases of toxemia of pregnancy were classified according to the severity of the symptoms, the frequency of increased transaminase values was essentially the same in all 3 groups, namely, in 28 per cent ($\frac{5}{18}$) of Group 1, in 30 per cent ($\frac{6}{20}$) of Group 2, and in 25 per cent ($\frac{2}{8}$) of Group 3. *Thus no correlation was found between the frequency of increased transaminase activity and the severity of toxemia of pregnancy.*

The results obtained in the present investigation support the assumption (2) that the rise in SGO-T and/or SGP-T demonstrable in about one third of all cases of toxemia of pregnancy is due to liver disorders or damage. The transaminase values were high in 13 of the 46 cases of toxemia. In 9 of these 13 cases there was evidence of impaired liver function. *Thus 69 per cent of the patients with an increased transaminase value had symptoms of liver damage.*

On the other hand, no correlation could be demonstrated between increased transaminase activity and any other symptom, laboratory finding or clinical datum such as parity, birth-weight, weight of the placenta, severity of renal injury, or rise in blood pressure.

In some cases the rise in the SGP-T value was greater than the rise in SGO-T; in others only the SGP-T value was abnormally high. This is a characteristic feature of infectious hepatitis (4, 5, 14). The liver contains more GO-T than GP-T (4), so that a greater rise in SGP-T than in SGO-T activity argues against the theory that an increased transaminase value is simply a manifestation of hepatocellular damage (4). The actual cause of the increase in the activity of serum transaminase in toxemia of pregnancy is not yet properly understood.

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STUDIES OF THE DIURNAL VARIATION OF PLASMA 17-HYDROXYCORTICOSTEROIDS IN MAN*

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ABSTRACT

Studies are reported of the diurnal variation of plasma 17-OHCS concentration in normal subjects and in patients with various diseases. Only in clinical situations associated with alterations in consciousness or sleep pattern was there significant loss of the diurnal rhythm. The rate of removal of hydrocortisone from the plasma was the same at various times of the day, but the adrenal production of 17-OHCS in response to maximally stimulating doses of ACTH was less at night than at other times during the 24-hour period. Submaximal stimulation of the adrenal cortex with ACTH during the late afternoon and evening restored to normal the nocturnal adrenal response to ACTH. It is suggested that the diurnal variation of plasma 17-OHCS depends on variations in the rate of secretion of ACTH. The change in ACTH secretion apparently is controlled by a central nervous system mechanism of unknown character. Total reversal of sleep and activity in normal subjects results in reversal of the diurnal rhythm in plasma 17-OHCS and serum iron levels, and body temperature.

A DIURNAL rhythm in the excretion of 17-ketosteroids was originally described by Pincus (1). This phenomenon was considered by him to represent a cyclic variation in adrenal function. Other investigators have confirmed and amplified these observations, and it is known that the diurnal rhythm of adrenocortical function is reflected in blood leukocytes (2), urinary 17-hydroxycorticosteroids (3), and plasma 17-hydroxycorticosteroids (17-OHCS) (4, 5).

Previously (5), we have reported studies of the diurnal variation in the level of plasma 17-OHCS throughout the 24-hour period. It was observed that the lowest concentrations of these steroids usually were found be-

Received October 16, 1958.

* This work was performed in part under Contract #DA-49-007-MD-136 with the United States Army and Research Grants from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service.

tween midnight and 4:00 A.M., followed by a sharp rise, with peak concentrations between 6:00 and 8:00 A.M. Neither total blindness nor partial reversal of sleep patterns had a significant effect on this diurnal rhythm.

The present studies were designed to characterize the alterations produced in the diurnal variation of plasma 17-OHCS concentration by disease states, and to elucidate the mechanism of the diurnal variation in adrenal steroid levels.

MATERIALS AND METHODS

Normal subjects. Plasma 17-OHCS levels were measured at 8:00 A.M., 12:00 noon, 4:00 P.M., 8:00 P.M., 12:00 midnight and 4:00 A.M. in 11 normal subjects—6 females and 5 males. Including the data reported previously (4, 5), values for plasma 17-OHCS at different times of the day and the night were thus made available in 51 normal subjects.

Patients. Observations were made in 31 patients with various nonendocrine disease states. The clinical data are shown in Table 1. This group was selected to include patients who were acutely or chronically ill (with and without fever) but with normal sleep

TABLE 1. CLINICAL DATA IN 31 PATIENTS WITH VARIOUS DISEASES

Patient	Age & Sex	Diagnosis	Fever (°F.)	Sleep habits; state of consciousness	Duration of bed or chair rest	Character of diurnal variation in plasma 17-OHCS
E.A.	25 F	Aseptic meningoencephalitis	103.5°	Comatose	9 days	Irregular decrease
E.L.	47 F	Rheumatoid arthritis	None	Normal	20 days	Normal
E.C.	76 F	Senility	None	Normal	Weeks	Normal
C.N.	67 M	Hodgkin's disease	104°	Normal	11 days	Normal
M.V.	55 M	Chlorpromazine jaundice	100°	Normal	2 mos.	Irregular decrease
A.W.	41 F	Laennec's cirrhosis	100°	Normal	40 days	Normal
L.A.	42 M	Laennec's cirrhosis	100–101°	Normal	9 days	Normal
M.F.	55 F	Pulmonary tuberculosis	102–103°	Normal	19 days	Normal
M.G.	64 F	Cerebral thrombosis	None	Normal	9 days	Irregular decrease
S.A.	17 F	Biliary cirrhosis	100–101°	Intermittent sleep	9 days	Little change
C.C.	55 M	Acute glomerulonephritis	None	Restless; intermittent sleep	1½ mos.	Little change
J.B.	74 M	Carcinoma of the cecum	100.5°	Delirious	4 days	Less decrease than normal
E.S.	59 F	Chronic pyelonephritis	None	Intermittent sleep	3 days	Abnormal increase
V.J.	78 F	Carcinoma of the breast	101°	Semicoma	Months	Irregular
M.H.	58 F	Cerebral thrombosis; pyelonephritis	None	Semicoma	2 mos.	Irregular
H.T.	64 M	Bronchopneumonia	102°	Normal	1 day	Irregular
B.A.	31 M	Quadriplegia; poliomyelitis	None	Intermittent sleep	1 mo.	Abnormal increase
M.L.	41 F	Bronchial asthma	None	Normal	1 day	Abnormal increase
J.D.	96 M	Diabetes mellitus; senility	None	Normal	3 yrs.	Irregular
H.A.	57 M	Multiple sclerosis	None	Normal	1 yr.	Normal
L.L.	46 F	Unknown	None	Normal	10 yrs.	Normal
N.R.	64 M	Paraplegia	None	Normal	10 yrs.	Normal
R.E.	40 M	Dystonia musculorum deformans	None	Unknown	30 yrs.	Normal
M.C.	74 M	Fractured hip	None	Unknown	? yrs.	Normal
W.McK.	88 M	Rheumatoid arthritis	None	Unknown	Years	Irregular
F.L.	78 F	Cerebral atrophy	None	Unknown	Years	Irregular
T.F.	103 M	Senility	None	Unknown	Years	Irregular
B.C.	83 F	Rheumatic heart disease	101°	Comatose	10 days	Irregular
B.H.	21 M	Paraplegia	None	Normal	4 yrs.	Normal
L.A.	— M	Empyema	Unknown	Normal	?	Irregular
P.D.	87 F	Cerebral thrombosis	100°	Semicomatose	7 days	Irregular

habits, and those in whom the state of consciousness was abnormal. In addition, 3 patients with untreated thyrotoxicosis and 2 with untreated acromegaly were studied.

ACTH infusions. Adrenocorticotropin (ACTH) was administered intravenously to normal subjects from 12:00 midnight to 8:00 A.M., from 4 P.M. to 10:00 P.M., and from 8:00 A.M. to 2:00 P.M., so that adrenocortical capacity might be estimated (6) at different times of the day and the night. In the ACTH tests started at midnight, the infusion time was lengthened to eight hours in order to include the portion of the day in which an abrupt rise in the plasma 17-OHCS level usually occurs. A dose of 40 units of ACTH was given in eight hours at this time of the night, instead of 25 units in six hours used in the other infusions. In addition, adrenal capacity was measured from 12:00 midnight to 8:00 A.M. in 3 subjects subsequent to the constant infusion of 0.5 unit of ACTH from 5:00 P.M. to 11:00 P.M.

Hydrocortisone disappearance rates. The removal of hydrocortisone from plasma was estimated at different periods of the day using a dose of 1 mg. per Kg. of body weight (7). Adrenal production of 17-OHCS was calculated from the disappearance rate and the adrenal response to ACTH (8).

Sleep reversal studies. The diurnal variation of plasma 17-OHCS concentration was studied in 9 normal subjects (5 male and 4 female) before and after complete reversal of sleep and activity patterns for five and ten days, respectively. These adult subjects were hospitalized on the Metabolic Ward of the Salt Lake County General Hospital. All windows had been covered previously with plyboard and black curtains, to exclude extraneous light. Each subject was required to be in bed and, if possible, asleep from 8:00 A.M. to 4:00 P.M., and to be awake from 4:00 to 8:00 A.M. By closing the ward to visitors and other persons during the day, daytime sleep was accomplished by all of the subjects. A general hospital diet was used, with breakfast offered between 4:00 and 5:00 P.M., lunch at 12:00 midnight, and dinner at 7:00 to 8:00 A.M. During the period of sleep reversal, plasma samples for determination of 17-OHCS were obtained from each subject at 8:00 A.M. and 4:00 P.M. A complete 24-hour diurnal variation was measured on the day preceding, and on the last day of the reversal period. In order to obtain a measure of the influence of sleep reversal on nonadrenal dependent diurnal patterns (5), plasma iron levels and body temperatures were studied in all 9 subjects, before and after the sleep reversal was accomplished.

Plasma 17-OHCS levels were determined as described by Nelson and Samuels (9) and by Eik-Nes (10). Serum iron concentration was measured by the technique of Hamilton *et al.* (11).

RESULTS

Normal subjects

The plasma 17-OHCS levels at different times of the day and night in 51 normal persons are plotted in Figure 1. The great majority of these normal subjects showed a decreasing concentration of plasma 17-OHCS after 8:00 A.M., with the nadir between 8:00 P.M. and 4:00 A.M., followed by a maximum at 8:00 A.M. A summary of these data is shown in Table 2.

Constancy of the diurnal variation in normal subjects

In Figure 2 the plasma 17-OHCS values at 8:00 A.M. and 4:00 P.M. in 5 normal young adult male subjects on four consecutive days are shown. In

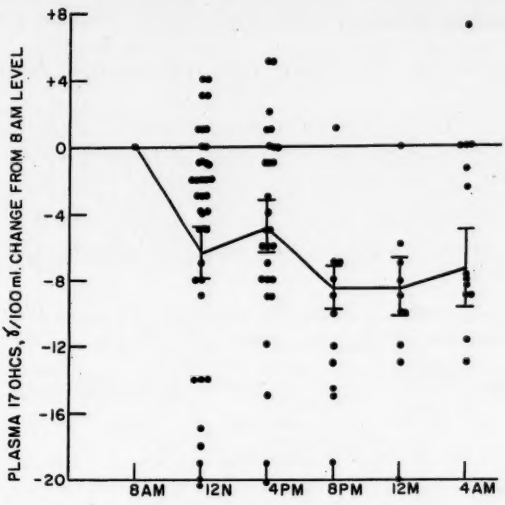

FIG. 1. Diurnal variation in the level of plasma 17-hydroxycorticosteroids in 51 normal subjects.

each instance, the direction of change was the same, with decreases of from 3.4 to 16.0 γ per 100 ml. of plasma. It is of interest that the days of least decrease in plasma steroid levels corresponded to periods of emotional stress in the daily work of each subject.

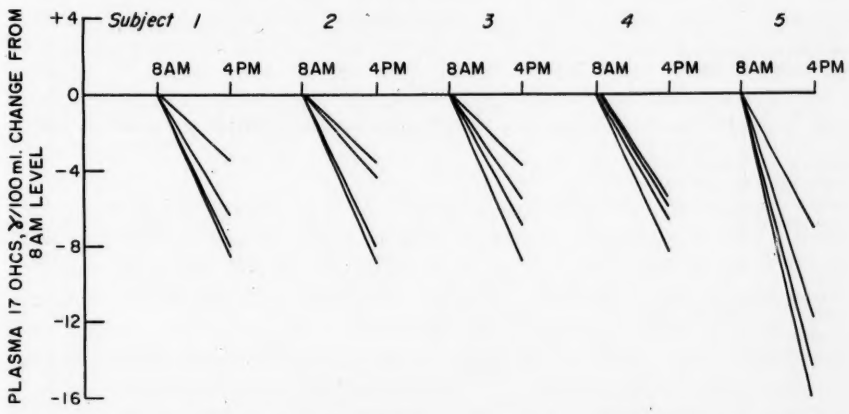

FIG. 2. Diurnal variation in the level of plasma 17-hydrocorticosteroids in 5 normal subjects on four successive days. Although the magnitude of change from the 8:00 A.M. level varies, in each instance the 4:00 P.M. values are lower than the 8:00 A.M. values.

Patients with various diseases

The data obtained in 31 patients with nonendocrine disorders are summarized in Table 2.

In contrast to the findings in normal subjects, a marked variation in both direction and magnitude of the change from the 8:00 A.M. value for plasma 17-OHCS was observed. For this group, the average change from 8:00 A.M. was smaller than in the normal subjects. It is noteworthy that the diurnal variation in patients with abnormal plasma 17-OHCS levels at

TABLE 2. DIURNAL VARIATION IN LEVEL OF PLASMA 17-OHCS
(γ PER 100 ML. DEVIATION FROM 8:00 A.M. LEVEL)

	No. of patients	17-OHCS; avge. 8:00 A.M. level (γ/100 ml.)	Change in 17-OHCS from 8:00 A.M. level at various times of day (γ/100 ml.)				
			12:00 noon	4:00 P.M.	8:00 P.M.	12:00 midnight	4:00 A.M.
Normal subjects	51						
Average		16.02	−6.4	−4.9	−8.5	−8.5	−7.4
σ		6.28	10.12	8.05	5.04	6.91	8.99
σ_m			1.42	1.13	0.71	0.97	1.26
Various diseases	31						
Average		16.96	−1.3	−1.5	−3.3	−3.6	−2.2
σ		12.14	6.99	7.58	7.71	9.02	6.19
σ_m			1.26	1.36	1.38	1.62	1.11
Patients with 8:00 A.M. values higher than 20 γ/100 ml.	7						
Average		32.00	−4.6	−4.8	−5.5	−8.4	−3.2
σ		35.08	5.41	8.21	9.67	13.91	4.01
σ_m			2.04	3.10	3.65	5.26	1.52
Patients with fever and a normal state of consciousness	12						
Average		34.6	−3.7	−3.2	−3.4	−3.3	−3.4
σ		18.85	0.48	3.13	6.43	7.91	2.43
σ_m			0.138	0.903	1.85	2.28	0.701
Patients with altered state of consciousness or abnormal sleep pattern	10						
Average		22.3	−1.3	+2.3	−0.3	+0.5	−0.2
σ		20.28	6.49	6.49	7.13	11.88	5.40
σ_m			2.05	2.05	2.25	3.76	0.736
Patients with normal state of consciousness and sleep pattern without fever	9						
Average		13.4	−0.2	−5.7	−4.9	−8.3	−1.3
σ		6.68	8.21	7.03	8.94	8.48	7.12
σ_m			2.74	2.34	3.13	2.83	1.37
Patients inactive more than one year	10						
Average		14.2	−3.0	−1.9	−7.0	−9.0	−4.6
σ		5.46	6.04	10.18	5.80	7.04	4.19
σ_m			1.91	3.22	1.83	2.23	1.33

8:00 A.M. closely resembled the normal curve (Table 2), although the deviations around the means were much greater in this group.

In an attempt to identify the reasons for the marked variability of the diurnal variation of plasma 17-OHCS concentration found in disease, the data for various patients were subdivided according to the presence or absence of fever, to altered states of consciousness or sleep patterns, and to prolonged inactivity. These data are depicted in Figure 3. The curves most closely resembling the normal diurnal variation in shape and degree of change were those of the patients with a normal state of consciousness

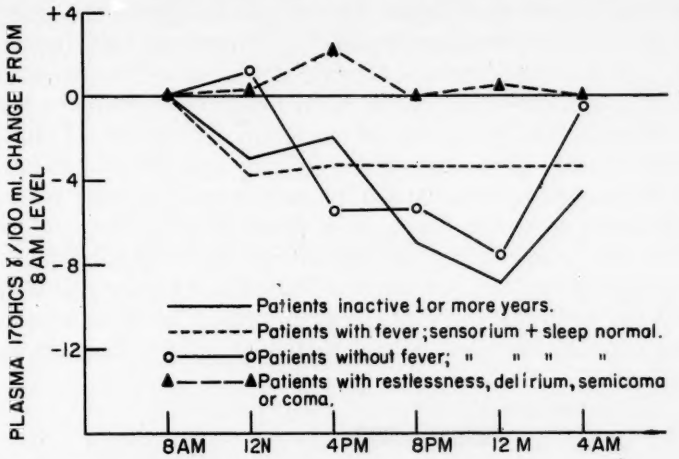

FIG. 3. Diurnal variation of plasma 17-hydroxycorticosteroid concentration in 4 groups of sick patients. Note that in patients with restlessness, delirium, semicoma, or coma there was little or no diurnal variation of the plasma 17-hydroxycorticosteroid level. In patients with or without fever, but with a normal sensorium and sleep pattern, as well as in inactive patients, there was a diurnal pattern which closely resembled the normal.

and a normal sleep pattern. It is of particular interest that in patients who had been bed-ridden or chair-ridden for one or more years, there was virtually a normal diurnal variation. The patients who had fever showed a smaller diurnal variation which was in the same general direction as that found in the normal subjects. Individual patients with altered sleep pattern or altered consciousness showed unpredictable variations in the direction and degree of change from the 8:00 A.M. values. The average for this group was little different from the 8:00 A.M. value.

In the 3 patients with thyrotoxicosis and the 2 patients with acromegaly, there was a completely normal diurnal variation of plasma 17-OHCS concentration.

Sleep reversal studies

In order to define further the factors accounting for the diurnal variation of the plasma 17-OHCS level and to delineate the limits of constancy of this phenomenon, 2 groups of normal adults were subjected to reversal of their day:night sleep and work pattern. The first group consisted of 5 male medical students whose sleep-work patterns were reversed in the manner described previously for five days during a school vacation. Only 4 of the male subjects completed the reversal period. The second group consisted of 4 normal female nurses, who performed general ward duty on the night shift during a ten-day reversal period. All 4 nurses completed the study.

In contrast to the findings reported previously in night workers who actually had only partial reversal of day:night activity, the diurnal pattern of plasma 17-OHCS concentration showed distinct inversion by the end of the reversal period. Two kinds of change in the plasma 17-OHCS level occurred. As early as the fourth day in some subjects and as late as the eighth day in others, the 8:00 A.M. plasma values decreased markedly, in some instances to values below those measurable by the methods used. The 4:00 P.M. values did not change initially, but rose subsequently. The data for the 2 groups are combined in Table 2 and Figure 4. It is apparent that reversal occurred, and that the magnitude of the change during sleep was less after the reversal. It is of particular interest that the period of

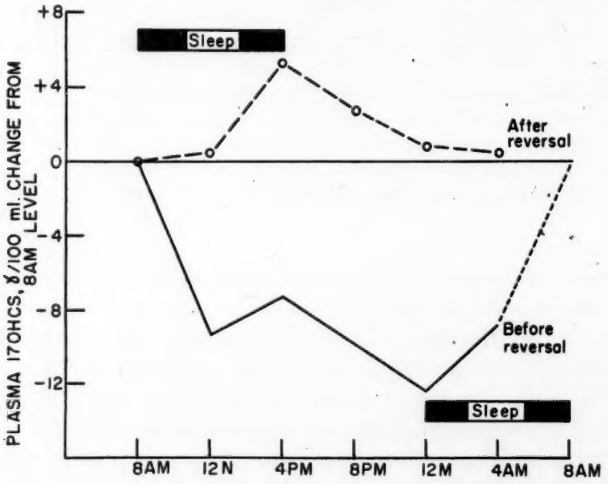

FIG. 4. Diurnal variation of plasma 17-hydroxycorticosteroid concentration in 8 subjects before and after reveral of sleep and activity. Note that in each instance, in spite of the reversal of the diurnal variation, the period of abrupt rise in the steroid level began *during* the period of sleep.

abrupt rise in the plasma steroid level occurred *during* the sleep period, both before and after the reversal period.

With reference to the other diurnal variations measured during the sleep reversal studies, the body temperature and the serum iron level were inverted by the end of the reversal period. Body temperature and serum iron levels are shown in Figure 5.

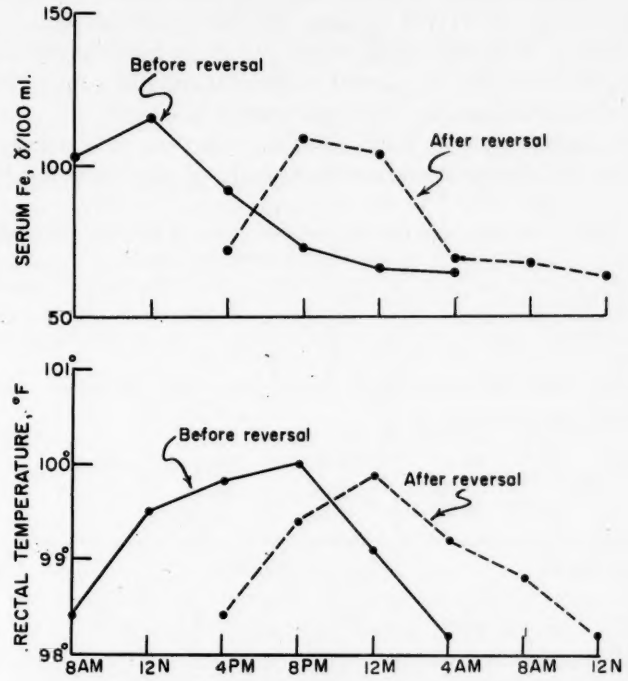

FIG. 5. Diurnal variation of body temperature and serum iron level in 8 subjects before and after reversal of sleep and activity.

Hydrocortisone disappearance rate

The rate of disappearance of infused hydrocortisone from the plasma of 6 normal subjects was measured at 8:00 A.M. (the usual time of a high plasma 17-OHCS level), and in 4 subjects from 12:00 midnight to 6:00 A.M. (the time of change from the lowest to the highest plasma 17-OHCS level). Similar studies were carried out in 6 normal subjects from noon to 6:00 P.M. The half-time of hydrocortisone in the plasma at 8:00 A.M. was 132 minutes; at noon, 133 minutes; and at midnight, 135 minutes. There is no significant difference between these values.

ACTH infusions

In Table 3 and Figure 6 are shown the effects of ACTH infusions starting at 8:00 A.M., 4:00 P.M. and 12:00 midnight. Whereas the peak level of plasma 17-OHCS achieved in response to the intravenous administration of ACTH was the same during the 8:00 A.M. and 4:00 P.M. infusions, the levels reached from midnight to 8:00 A.M. were considerably lower than those in the preceding experiments. Adrenal production of 17-OHCS, calculated by the use of ACTH infusion and the hydrocortisone disappearance rate for the respective times of day (8), were: for infusions starting at 8:00 A.M., 15.3γ per 100 ml. per hour; at 4:00 P.M., 15.5γ per 100 ml. per hour; and at 12:00 midnight, 10.5γ per 100 ml. per hour.

The low adrenal capacity for 17-OHCS production at night conceivably could be the result of an alteration in adrenal responsiveness resulting from

TABLE 3. PLASMA 17-HYDROXYCORTICOSTEROID LEVELS* IN RESPONSE TO ACTH AT DIFFERENT TIMES OF THE DAY

Time of day	Hours				
	0	2	4	6	8
8:00 A.M.–2:00 P.M. (N = 5)					
Average	11.4	29.0	38.5	43.6	
σ	5.01	10.25	10.18	11.48	
σ_m	2.24	4.58	4,55	5.13	
4:00 P.M.–10:00 P.M. (N = 4)					
Average	7.3	28.8	37.0	44.8	
σ	2.62	6.33	4.31	2.78	
σ_m	1.31	3.17	2.16	1.39	
12:00 M.–8:00 A.M. (N = 6)					
Average	5.5	20.1	29.5	30.4	32.8
σ	3.86	4.49	2.96	3.94	5.16
σ_m	1.58	1.83	1.21	1.61	2.11
12:00 M.–8:00 A.M. (after ACTH, 0.5 unit) (N = 3)					
Average	18.4	32.0	40.5	44.5	47.0
σ	2.97	0.82	0.50	4.64	9.63
σ_m	1.71	0.47	0.29	2.68	5.56

* Expressed as γ 17-hydroxycorticosteroid per 100 ml. plasma.

decreased secretion of ACTH during the preceding daytime hours. To test this hypothesis, 3 normal subjects were given a six-hour infusion of ACTH from 5:00 P.M. to 11:00 P.M. The dose used was 0.5 unit of ACTH, an amount we previously found sufficient to stimulate the adrenal gland submaximally (12). One hour after the infusion was stopped, a maximal-response ACTH test was begun (40 units of ACTH intravenously over eight hours, from 12:00 midnight to 8:00 A.M.). When the test was carried

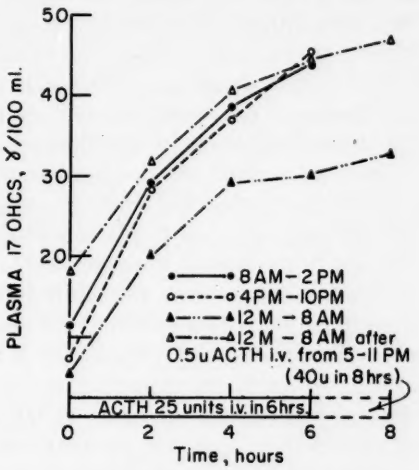

FIG. 6. Plasma 17-hydroxycorticosteroid response to maximal stimulation with ACTH at different times of the day. Note that the decreased adrenal capacity at night returned to normal after submaximal stimulation with ACTH during the preceding hours.

out in this fashion, adrenal production of 17-OHCS from 12:00 midnight to 8:00 A.M. was 16.1γ per 100 ml. per hour. This was virtually the same rate that was found during the other times of the day, and one and a half times the rate of adrenal production during these hours without prior ACTH stimulation. The ACTH response curves under these 2 circumstances are included in Table 3 and Figure 6. It is recognized that statistical analysis of such small series is of questionable significance. However, the derived values shown in Table 3 demonstrate the consistent nature of these responses to ACTH.

DISCUSSION

There are several possible explanations for the diurnal variation in the concentration of plasma 17-OHCS. These include 1) different rates of removal of hydrocortisone from the plasma at different times of the day, 2) variation in adrenal responsiveness of ACTH, and 3) changes in the rate of secretion of ACTH in response to a centrally located controlling mechanism.

Our finding that hydrocortisone is removed from the plasma at the same

rate, irrespective of the time of day or night, is against the first of these possibilities. In addition, patients with thyrotoxicosis (in whom hydrocortisone is removed from the plasma at a rate greater than normal (13)) exhibit a normal diurnal variation of adrenal steroids.

Forsham *et al.* (14) proposed that there is a nocturnal change in the responsiveness of the adrenal to ACTH which accounts for the diurnal variation. This concept was based on studies of the urinary excretion of 17-OHCS after the administration of ACTH gel at different times of the day. Our finding of a *smaller* rise in the level of plasma 17-OHCS in response to maximal stimulation of the adrenal with intravenous ACTH from 12:00 midnight to 8:00 A.M. demonstrates such a change. However, the *decreased* nocturnal adrenal capacity which we have demonstrated could hardly account for a rapid *increase* in secretion rate which must occur in order to produce the rise in plasma levels during this period.

Several experimental findings support the third explanation, *i.e.*, that the diurnal variation in plasma 17-OHCS concentration is dependent on changes in the rate of secretion of ACTH in response to a centrally located controlling mechanism. Our studies have demonstrated the character and reproducibility of the diurnal variation of the plasma 17-OHCS level in normal subjects and in various pathologic states. Only patients with altered states of consciousness or sleep regularly failed to show significant diurnal variation in the usual 17-OHCS pattern. Instead, the pattern was similar to those found in patients with severe brain damage, as previously reported from our laboratory by Eik-Nes and Clark (15). This close association of altered consciousness or sleep with abnormal diurnal variation of the plasma 17-OHCS level suggests a central site of control of this rhythm. In addition, our finding that prior submaximal stimulation of the adrenal cortex with ACTH increases the adrenocortical capacity to normal during the nocturnal period, is good evidence that the diurnal change in adrenocortical capacity is itself dependent on changes in ACTH secretion.

It seems reasonable, therefore, that the mechanism controlling the diurnal variation of plasma 17-OHCS concentration is centrally located and is related to the secretion of ACTH. The reversal studies described in this report demonstrate that one can change the time of occurrence of the events initiating ACTH secretion, but the stimulus for those events cannot be identified from our data. The results of these reversal studies, as well as the previously reported normal diurnal pattern in blind subjects (5), exclude stimulation from sunlight as an important initiating factor. A fundamental characteristic of the diurnal variation of plasma 17-OHCS concentration is the abrupt rise which occurs *during* the sleep period, a phenomenon observed in individual subjects during periods of normal or reversed sleep and activity patterns. However, it was not possible to alter sleep with-

out altering activity. Therefore, although in various diseases resulting in prolonged inactivity the direction and degree of diurnal rhythm of the plasma 17-OHCS level was found to be normal, the relative importance of sleep versus activity is as yet uncertain.

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URINARY EXCRETION OF 17-KETOSTEROIDS FOLLOWING RECTAL INFUSION OF CORTISOL

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ABSTRACT

Urinary 17-ketosteroid excretion patterns were compared, following rectal and oral administration of cortisol to a man with quiescent ulcerative colitis. Steroid conjugates extracted from the urine were hydrolyzed to yield glucuronide and sulphate fractions, and the free steroids separated on alumina. Individual compounds were identified by paper chromatography and by infrared spectroscopy. Rectal infusions of cortisol produced a 100-fold increase in urinary 11β-hydroxyetiocholanolone excretion (from 92 μg. to 10,350 μg. daily), and a 25-fold increase in 11β-hydroxyandrosterone excretion (from 98 μg. to 2465 μg. daily). Other 17-ketosteroids were hardly changed. Oral cortisol had little effect on urinary 17-ketosteroid excretion. The increased excretion of 11β-hydroxyetiocholanolone and 11β-hydroxyandrosterone after rectal infusion of cortisol did not occur when the bacterial activity of the gut was reduced by giving neomycin orally.

IN 1957, Nabarro and associates (1) showed that rectal infusions of cortisol could produce a considerable increase in the urinary excretion rate of 17-hydroxycorticosteroids (17-OHCS) and 17-ketosteroids (17-KS) in patients with ulcerative colitis. One patient (Case 5 (1)) showed a remarkable rise in 17-KS excretion, up to 60 mg. per day. A further course of treatment with rectal infusions of cortisol has been given to this man and the compounds involved in the increased urinary 17-KS excretion have been identified. Neomycin, given by mouth, was found to suppress the increase in 17-KS excretion.

CLINICAL

The patient, a man aged 52, had recovered from an acute attack of ulcerative colitis five weeks previously. His bowels moved two or three times a day, the stools being semi-formed but without blood or mucus. The sigmoidoscopic appearance of the rectal and colonic mucosa had reverted to normal, although x-ray examination after a barium enema still demon-

Received September 15, 1958.

strated the changes of ulcerative colitis. Culture of the feces on desoxycholate citrate medium did not produce any pathogenic organisms.

Cortisol was given per rectum as a 1 per cent solution of the free alcohol dissolved in a mixture of glycols (Roussel skin lotion). A 20-ml. portion of this solution (containing 200 mg. cortisol) was diluted in 120 ml. of physiologic sodium chloride and infused into the rectum during a 15-minute period, starting at 7:00 P.M.. The enema was retained for at least twelve hours. Urine collections (without preservative) were made from 6:00 A.M. to 6:00 A.M. The completeness of the 24-hour collection was checked by daily creatinine estimation.

Oral cortisol (200 mg.) was administered as 10 tablets at 7:00 P.M.

The course of treatment is shown in Figure 1. Urine was examined before, during and after rectal infusion of cortisol, and comparison made with

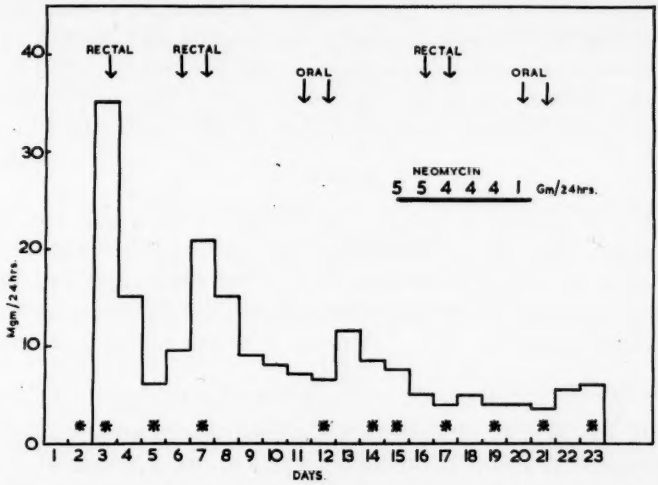

FIG. 1. Showing the plan of the investigation and the total 17-ketosteroid output in the urine. The arrows indicate rectal infusions and oral administration of cortisol (200 mg.). The asterisks indicate the days on which detailed analysis was performed.

the urinary 17-KS output after oral administration of cortisol. Rectal infusions of cortisol were also given during treatment with neomycin (23 Gm. orally for a period of five days).

Although urines were collected daily, detailed analysis was carried out on one collection only from each phase of the study (see Fig. 1).

CHEMICAL METHODS

The methods used have been described in detail elsewhere (2). Only minor modifications were made. The procedure involved (*a*) extraction of steroid conjugates from the

urine (3), (*b*) independent hydrolysis of steroid glucuronides and sulphates, (*c*) chromatography of these fractions on alumina using a gradient elution system, (*d*) accurate estimation of individual compounds, and (*e*) identification of compounds by paper chromatography and, when possible, infrared spectroscopy.

To determine material not hydrolyzed by milder procedures, the aqueous solution remaining after sulphate hydrolysis and ether extraction, together with the alkali washings of the ether extract, was acidified (HCl 10 per cent v/v) and subjected to hydrolysis by boiling for ten minutes. The cooled solution was extracted with benzene.

All fractions obtained after hydrolysis were separated into ketones and non-ketones (4). This step is essential when pigment is present; otherwise there may be difficulties in subsequent colorimetric determination and in paper chromatography.

The 17-KS freed by β-glucuronidase hydrolysis of conjugates were chromatographed on 6 Gm. of Al_2O_3, and those freed by mild acid hydrolysis, on 4.5 Gm. of Al_2O_3 (internal diameter of column in both cases, 0.6 cm.). Although 4.5 Gm. of Al_2O_3 does not provide as good a separation as 6 Gm., the running speed is faster, and experiments showed that with a 4.5-Gm. column there was sufficiently good separation of the sulphate fraction.

Material obtained by hot acid hydrolysis was chromatographed only on paper.

The paper chromatography systems used were those of Bush (A and B1 (5)).

As a guide during subsequent fractionation, the total 17-KS content of each 24-hour urine specimen was estimated by a rapid method described previously (6).

RESULTS

Total 17-KS excretion

As shown in Figure 1, rectal infusions of cortisol produced a considerable increase of total 17-KS excretion. This effect was suppressed by giving neomycin by mouth for two days beforehand. By contrast, cortisol given orally had little effect.

Fractionation of 17-KS

Most of the 17-ketosteroids were excreted as glucuronide, the proportion (average 87.9 per cent) being nearly the same throughout the period of study. It will be seen in Table 1 that total 17-KS excretion determined by hot acid hydrolysis was consistently higher (by an average of 36.9 per cent) than the excretion determined by adding the fractions obtained by other methods of hydrolysis. This has not been observed in other work using these methods, but the same observation has been made previously on this patient. Table 2 shows the loss incurred during fractionation of the conjugate extract of urine specimens obtained following rectal infusions of cortisol. Estimation of 17-KS at each stage of the fractionation procedure showed no loss either in the initial extraction of conjugates or in the Girard separations. The loss occurred after the glucuronides and sulphates had been hydrolyzed; only very small amounts of 17-KS were recovered from the remaining aqueous solution. If the loss had been due to incomplete hydrolysis of glucuronides, unhydrolyzed material (extracted by ether dur-

TABLE 1. DISTRIBUTION OF TOTAL 17-KETOSTEROIDS (MG./24 HRS.) IN THE URINES ANALYZED

K = Ketones, F = Cortisol, N = Neomycin

Day*	Period	Urine total (hot acid hydrolysis)	Conjugate extract			Total ketones
			Glucuronide (K)	Sulphate (K)	Aqueous (K)	
2	Control	Not det'd	4.0	0.5	0.2	4.7
3	Rectal F	35.4	17.8	1.6	0.7	20.1
5	Rest	5.9	3.4	0.7	0.1	4.2
7	Rectal F	20.7	9.2	1.2	0.3	10.7
12	Oral F	7.0	3.4	0.3	0.2	3.9
14	Rest	8.6	4.0	0.4	0.1	4.5
15	Rest+N	7.3	4.0	0.4	0.1	4.5
17	Rectal F+N	4.1	2.4	0.2	0.2	2.8
19	Rest	3.9	2.4	0.1	0.1	2.6
21	Oral F	3.5	2.5	0.2	0.1	2.8
23	Rest	6.2	3.3	0.4	0.1	3.8

* See Figure 1.

ing sulphate hydrolysis) would have passed into the alkali washings which had been added to the aqueous solution before hot acid hydrolysis (see Methods). If a new conjugate had been present which remained unaffected by β-glucuronidase and by mild acid hydrolysis, it should have appeared in either the ether or the aqueous fraction.

Analysis of the glucuronide fractions

A 1-mg. aliquot of 17-KS from each glucuronide fraction was chromatographed on alumina, and the daily excretion of seven 17-KS determined

TABLE 2. LOSS OF 17-KETOSTEROID DURING FRACTIONATION OF URINARY CONJUGATE EXTRACT (MG./24 HRS.)

K = Ketones

Urine total (hot acid) hydrolysis	Conjugate extract			Total ketones	Per cent of urine total
	Glucuronide (K)	Sulphate (K)	Aqueous (K)		
57.0*	26.7	2.4	1.1	30.2	53.0
35.4	17.8	1.6	0.7	20.1	56.7
20.7	9.2	1.2	0.3	10.7	51.7

* Upper row of values relates to a previous investigation (1).

(Table 3). The efficiency of the chromatographic separation is shown by the excretion patterns illustrated in Figure 2.

(*a*) *11-deoxygenated 17-KS.* Normally these compounds account for most of the total 17-KS excretion. The control urine from our patient was no exception, 84.4 per cent of the 17-KS glucuronides being 11-deoxygenated compounds (Table 3). Androsterone and etiocholanolone, the chief urinary 11-deoxygenated 17-KS, were present in normal amounts. After rectal infusion of cortisol, the excretion rate of both of these compounds decreased. Expressed as a percentage of the total 17-KS glucuronide fraction, androsterone output fell from 26.5 to 4.2 per cent, and etiocholanolone output from 53.2 to 8.9 per cent. During the following rest periods, and also when neomycin was given with the rectal infusions of cortisol, the proportions of androsterone and etiocholanolone returned to the control values, although the absolute level of excretion remained lower than at first (Table 3).

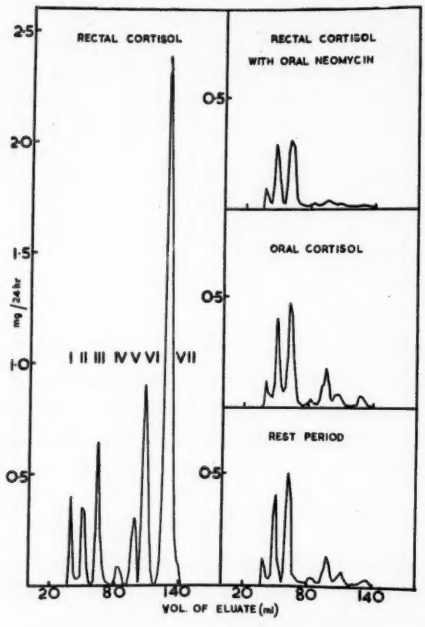

FIG. 2. Urinary 17-ketosteroid excretion patterns. Left—day 3; lower right—day 5; middle right—day 12; top right—day 17 (see Fig. 1).

The material first eluted from the column (Peak I, Fig. 2) contained only small amounts of 11-deoxy 17-KS. On paper chromatography (Bush A system (5)) 5α-androstan-3:17-dione and/or its 5β-isomer were tentatively identified. Final identification was not possible because there was insufficient material for infrared spectroscopy, and the two compounds are not separable by the chromatographic systems used. No dehydroepiandrosterone was found.

(b) *11-oxygenated 17-KS.* These formed 15.6 per cent of the 17-KS glucuronide fraction in the control urine. Four of these compounds were identified: 11-ketoandrosterone, 11-ketoetiocholanolone, 11β-hydroxyandrosterone, and 11β-hydroxyetiocholanolone. Following rectal infusion of cortisol, a remarkable increase in the urinary excretion rate of the last 2 compounds was observed; the output of 11β-hydroxyandrosterone rose from 98 to 2465 μg. daily, and the output of 11β-hydroxyetiocholanolone

TABLE 3. URINARY EXCRETION OF INDIVIDUAL 17-KETOSTEROIDS IN THE GLUCURONIDE FRACTION (μG./24 HRS.)
F = Cortisol, N = Neomycin

Compound	Control period	Rectal F	Rest	Rectal F	Oral F	Rest	Rest+N	Rectal F+N	Rest	Oral F	Rest
	Day* 2	Day 3	Day 5	Day 7	Day 12	Day 14	Day 15	Day 17	Day 19	Day 21	Day 23
Dione fraction	180 (4.7)†	587 (3.6)	163 (5.0)	424 (4.6)	135 (4.1)	195 (5.1)	261 (6.7)	136 (5.9)	150 (6.5)	206 (8.5)	196 (6.1)
Andosterone	1,017(26.5)	694 (4.2)	772(23.6)	1,061(11.6)	633(19.1)	869(22.7)	790(20.3)	724(31.4)	678(29.2)	578(23.8)	842(26.3)
Etiocholanolone	2,038(53.2)	1,464 (8.9)	1,437(43.9)	2,310(25.3)	1,361(41.0)	1,800(46.9)	2,049(52.7)	1,083(47.0)	1,154(49.7)	1,320(54.5)	1,837(57.4)
11-Ketoandrosterone	92 (2.3)	191 (1.2)	87 (2.7)	122 (1.3)	85 (2.6)	92 (2.4)	118 (3.0)	51 (2.2)	66 (2.8)	48 (2.0)	59 (1.8)
11-Ketoetiocholanolone	316 (8.3)	644 (3.9)	545(16.6)	652 (7.1)	781(23.5)	660(17.2)	385 (9.9)	154 (6.7)	91 (3.9)	139 (5.7)	86 (2.7)
11β-Hydroxyandrosterone	98 (2.6)	2,465(15.0)	178 (5.4)	1,129(12.3)	162 (4.9)	126 (3.3)	180 (4.6)	54 (2.3)	95 (4.1)	63 (2.6)	101 (3.2)
11β-Hydroxyetiocholanolone	92 (2.3)	10,350(63.5)	93 (2.8)	3,450(37.7)	162 (4.9)	92 (2.4)	106 (2.7)	103 (4.5)	89 (3.8)	68 (2.8)	81 (2.5)

* See Figure 1.
† Values in parenthesis denote percentage composition.

increased about 100-fold, from 92 to 10,350 μg. daily. The latter compound accounted for 63.5 per cent of the total 17-KS glucuronides excreted on day 3, and 37.7 per cent on day 7. When oral neomycin was given at the same time as rectal cortisol, the excess excretion of both these compounds was suppressed. Oral cortisol did not influence the excretion rate of either compound significantly.

The output of 11-ketoetiocholanolone, the main 11-oxygenated 17-KS of the control urine, was slightly increased by both rectal and oral administration of cortisol. Neomycin appeared to suppress the small increase following rectal cortisol.

The excretion of 11-ketoandrosterone, always small, averaged only 2.3 per cent of the glucuronide fraction. A slightly increased excretion was seen following rectal cortisol, and this appeared to be suppressed by neomycin. Oral cortisol was without effect.

Analysis of the sulphate fraction

Quantitative estimation of the compounds present in the sulphate fraction was possible only in a few urine specimens, because of the small amounts present. Table 4 shows that 11-oxygenated 17-KS sulphate excretion rose after rectal infusion of cortisol, although the amounts were still small. Oral cortisol and rectal cortisol with oral neomycin had no detectable effect on the excretion rate of these compounds. The output of dehydro-

TABLE 4. URINARY EXCRETION OF INDIVIDUAL 17-KETOSTEROIDS IN THE SULPHATE FRACTION (μG./24 HRS.)

F = Cortisol, N = Neomycin

Compound	Control period	Rectal F	Rest	Oral F	Rest	Rest +N	Rectal F+N	Rest	Oral F	Rest
	Day* 2	Day 3	Day 5	Day 12	Day 14	Day 15	Day 17	Day 19	Day 21	Day 23
Dehydroepiandrosterone	240	272	Not Identified	49	75	62	21	Identified†	Identified	70
Androsterone	85	84	Identified	Identified	32	34	Identified			23
Etiocholanolone	161	197	Identified	42	51	46	32	Identified	30	42
11-Ketoandrosterone	33	42	43	37		28	18		15	36
11-Ketoetiocholanolone		296								
11β-Hydroxyandrosterone		105								
11β-Hydroxyetiocholanolone		544								

* See Figure 1.

† "Identified" means identified by paper chromatography, but no quantitative estimation possible. 11-Ketoetiocholanolone was identified in all urines except that from day 5.

epiandrosterone, initially only 240 μg. daily, decreased to very low levels soon after the first rectal infusion of cortisol and remained low, averaging about 57 μg. daily. It is noteworthy that the fall in dehydroepiandrosterone excretion took place after the first rectal infusion of cortisol, and before oral cortisol had been given.

Aqueous fractions

Paper chromatography of these fractions (Bush A and B1) showed the presence of small amounts of compounds which were not identified.

DISCUSSION

The principal urinary 17-KS metabolites of orally administered cortisol and cortisone are 11β-hydroxyetiocholanolone and 11-ketoetiocholanolone (7–9). 11β-Hydroxyetiocholanolone may be the major 17-KS metabolite of both steroids (8, 9); in some instances cortisone is converted predominantly to 11-ketoetiocholanolone (10, 11). Burstein, Savard and Dorfman (8) showed that cortisol can be converted to cortisone *in vivo*, and in the experiments of Peterson *et al.* (12), a large part of administered cortisone was converted to cortisol.

In the present study, oral administration of cortisol (200 mg.) led to only a small increase in the excretion of 11-oxygenated 17-KS, and this was largely of 11-ketoetiocholanolone (Table 3).

When cortisol was given by rectal infusion, urinary 17-KS excretion was appreciably increased, largely due to increased excretion of a single compound, 11β-hydroxyetiocholanolone. Very little of this compound was excreted during the control period. After a single administration of 200 mg. of cortisol rectally, more than 10 mg. of this 11-oxygenated 17-KS was excreted. The urinary excretion of 11β-hydroxyandrosterone was also increased but to a lesser extent (2.5 mg. daily). 11-Ketoandrosterone and 11-ketoetiocholanolone were only slightly increased following rectal cortisol, although 11-ketoetiocholanolone may be excreted in appreciably greater amount after oral cortisol (8).

Nabarro *et al.* (1) considered that the increased urinary excretion of 17-KS following rectal infusions of cortisol might result from bacterial modification of the administered steroid. Their hypothesis was tested by reducing the bacterial activity in the gut with neomycin, which was given before and during a rectal infusion of cortisol. This resulted in a return to the normal excretion pattern (Fig. 2). There was no increased excretion of any 11-oxygenated 17-KS, nor did any real increase of 11-oxygenated 17-KS occur when, following neomycin, oral cortisol was again given.

It seems logical to assume that the large amount of 11β-hydroxyetiocholanolone excreted after rectal infusion of cortisol resulted from bacterial

action on the administered steroid. That bacteria and moulds can cause steroids to undergo oxidative and reductive changes has been known for many years, and it is now well established that these organisms can carry out metabolic conversions of steroids equally as diverse as those occurring in animal tissues. The side-chain of C_{21}-steroids can be removed by a variety of organisms, and this may be accompanied by dehydrogenation of ring A. Thus, Vischer and Wettstein (13) showed that deoxycorticosterone, when incubated with *Fusarium solani* gives rise to $\Delta^{1,4}$-androstadiene-3,17-dione. These authors thought, however, that the presence of a 17α-hydroxyl group prevented side-chain degradation, since from 11-deoxycortisol and from cortisone, no C_{19}-steroids were obtained. Instead, the corresponding 1-dehydro derivatives were formed. Peterson *et al.* (14), on the other hand, found that several species of *Penicillium* and *Aspergillus* degraded acetyl, α-ketol, and dihydroxy-acetone side-chains with equal ease, and that 17α-hydroxysteroids were not immune to degradation.

Both the double bond and the carbonyl group of conjugated 3-ketones can be reduced by anaerobic bacteria. Yeasts can also carry out this reduction, but again, evidence has been presented which suggests that the presence of 17α-hydroxyl, or 21-hydroxyl groups may prevent the reaction (15).

Nothing is known of the organisms or the reactions involved in the conversion of cortisol to 11β-hydroxyandrosterone and 11β-hydroxyetiocholanolone in the gut, which it is assumed took place after rectal infusion of the steroid in our patient. Metabolism of infused cortisol appeared to be rapid; Peterson *et al.* (16) found that, when incubated with feces, cortisol was completely metabolized after four hours. The predominance of 5β-17-KS over 5α compounds agrees with the findings of Burstein *et al.* (8), who administered cortisol orally. The percentage conversion of cortisol to 5β-17-KS after rectal infusion (5 per cent) was higher than these authors found after oral administration.

After giving neomycin, the excretion of 11-oxygenated compounds remained low, and oral cortisol caused a negligible increase. Even before giving neomycin the increase was small, but eliminating bacteria from the intestine appears to have influenced the metabolism of oral as well as of rectal cortisol.

Following rectal infusion of cortisol, the decreased excretion rate of dehydroepiandrosterone, androsterone and etiocholanolone suggests an inhibitory effect on the adrenal gland, an effect maintained throughout the period of study.

Acknowledgments

We are grateful to Dr. J. D. N. Nabarro for his advice and encouragement, and for permission to study his patient; to the patient himself for volunteering to come into

the hospital for this study; and to the Nursing Staff for their unfailing help. We are also grateful to Sir Charles Dodds for his interest.

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THE INFLUENCE OF THYROID HORMONES ON METABOLISM IN PSYCHIATRIC DISORDERS. I. THE EFFECT OF 3:5:3′-TRIIODOTHYRONINE ON CALCIUM AND PHOSPHORUS METABOLISM IN PSYCHIATRIC PATIENTS*

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ABSTRACT

The effect of the intravenous and oral administration of 3:5:3′-triiodothyronine on calcium and phosphorus metabolism was studied in a series of 13 psychiatric patients. The metabolic observations in 3 schizophrenic patients are presented in detail, with reference made to the 10 other patients similarly studied. In these 3 patients the loss of phosphorus resulting from the administration of the hormone corresponded to the amount expected on the basis of nitrogen and calcium balance determinations. This observation is at variance with findings in myxedema. In the latter condition, triiodothyronine induced a loss of phosphorus exceeding the amount theoretically anticipated, indicating the breakdown of phosphocreatine and an increased rate of turnover of adenosinetriphosphate. In certain schizophrenic patients, triiodothyronine induced a negative calcium balance, primarily due to increased fecal loss of calcium. In other schizophrenic patients, no effect on calcium balance was observed and, at times, calcium retention was increased. The appearance of evident strong emotions corresponded in time to the induction of negative calcium balances in certain patients receiving triiodothyronine. The psychopathologic and biologic implications of these observations are discussed.

INTRODUCTION

THE overall purpose of our current research program is the exploration of possible relationships between thyroid function and mental diseases. Our specific aims include 1) determination of the influence of thyroid hormones and their analogues on emotions and psychopathologic states, and 2) investigation of the metabolic effects of thyroid hormones in psychiatric

Received August 1, 1958.

* Presented at the Annual Meeting of the American Goiter Association, San Francisco, California, June 17–19, 1958.

patients. We have already published preliminary reports on the type of psychologic changes observed in patients receiving triiodothyronine (1, 2). It is the purpose of this paper to present the observations which we have made regarding the effects of 3:5:3′-triiodothyronine on calcium and phosphorus metabolism in patients with psychiatric disorders, in particular schizophrenic reaction states.

There is considerable evidence in the literature that alterations in thyroid function may be associated with emotional changes. In patients with hyperthyroidism, for example, hostility and lability of mood are commonly seen. Patients with myxedema may present a variety of psychopathologic changes, labeled by some as the "psychoses of myxedema." In 1949, a report (3) on "Myxoedematous Madness" described 14 patients admitted to a psychiatric hospital for conditions characterized by confusion and apathy often associated with mood changes, hallucinations, and delusions of persecution. The confusional features usually cleared when the patients were restored to a euthyroid state; the delusions occasionally disappeared but often persisted after treatment. This is in keeping with the report (4) of the special Committee on Myxoedema appointed by the Clinical Society of London in 1883, which described marked retardation and sluggishness of mental processes among patients with untreated myxedema. They also described as a prominent feature, "irritability frequently alternating with periods of placidity." It was reported that delusions and hallucinations with pronounced manifestations of suspiciousness and self-depreciation occurred in nearly half the cases, mainly when the disease was in an advanced stage.

Such observations led to the investigation of thyroid function in disorders that were primarily psychiatric. For example, Bowman (5) and Hoskins (6) described a lowering of the basal metabolic rate among schizophrenic patients. Hoskins stated that the lowest of many basal metabolic rates in a large series of male patients averaged 19 per cent below the accepted standard. These authors also reported that schizophrenic patients appeared relatively resistant to large doses of desiccated thyroid. Brody and Man (7) reported normal serum protein-bound iodine levels among such patients. Cranswick (8) found that the thyroidal uptake of radioactive iodine in a majority of 31 schizophrenic patients was higher than that in controls. This observation was modified by the findings of Reiss (9) that among a mixed diagnostic group of psychiatric patients the thyroidal uptake of radioactive iodine was often unusually high or low, and returned to normal only when the patients had responded favorably to various therapies. Gjessing (10) and Gornall (11) applied metabolic balance studies to the investigation of psychiatric disorders, detecting wide fluctuations in nitrogen retention and excretion associated with recurrent

phases in periodic confusional and catatonic reactions. They were able to interrupt these recurrent patterns by the administration of large doses of thyroid hormones. A review of such studies suggested to us that, although the production of thyroid hormones may be normal in schizophrenic patients, the utilization of these hormones may be defective.

An important relationship between brain function and thyroid metabolism was established by Tata (12), who demonstrated that cerebral cortical tissue was capable of metabolizing labeled thyroxine to 3:5:3′-triiodothyronine and to tetraiodothyropropionic acid and/or tetraiodothyroacetic acid, followed by deiodination to triiodothyropropionic acid and/or triiodothyroacetic acid and finally to iodide. In 1953, Rawson and associates (13) described the effect of triiodothyronine on nitrogen and electrolyte metabolism in a patient with myxedema. In this study, although a very slight and probably insignificant increase in calcium loss was observed following the intravenous administration of triiodothyronine, a marked increase in urinary phosphorus was seen. This loss of phosphorus exceeded that which could be accounted for by the loss of nitrogen and calcium. Hoagland, Rinkel and Hyde (14) observed that schizophrenic patients lost less urinary phosphorus than did nonschizophrenic controls at rest, and that the rise of urinary phosphorus following stimulation with ACTH was significantly greater in schizophrenic patients than in normal controls. Therefore, we concluded that it would be of value to compare the effect of triiodothyronine on electrolyte metabolism in schizophrenic patients with the observations made by Rawson *et al.* in myxedema.

METHOD

These studies were carried out on the metabolic unit of the Payne Whitney Psychiatric Clinic. The 13 patients selected for study represented well-defined psychopathologic states, and were free of known physical disorders, in particular endocrine dysfunctions. Dietary intakes were constant throughout the entire period of study. Urines and stools were collected and analyzed for nitrogen, calcium; phosphorus and potassium content, according to accepted methods (13). Urinary creatine and creatinine determinations were made—the latter to assure accuracy of urine collections.

In the 3 patients who will be discussed in detail, triiodothyronine was administered at the end of control periods, first intravenously in doses of 500–800 micrograms, and then orally in dosages of 100–300 micrograms daily for periods of two to three weeks.

Continuing observations of the emotional and psychopathologic states of these patients were carried out by the research psychiatrists, individual psychotherapists, and the psychiatric nursing staff on the unit. Careful attention was paid to describing the quality and intensity of emotional changes in the patients under investigation. Behavioral graphs were used to supplement individual observations and subjective reporting by patients. Hostile emotions were considered to be present when a subject verbally, through facial expression and gesture, or in group relationships indicated the presence of anger or resentment, overtly experienced and outwardly directed. Sexual unrest was considered to be present when there was evidence of sexual stimulation of a homosexual

or heterosexual type, through verbal expression or in behavior. Such states of mild or intense excitement were observed in dreams, conscious phantasies surrounding sexual activity, or erotic behavior in group relationships which could be observed in bodily appearance, dress, conversation and activity.

OBSERVATIONS

Patient A (PWC-4)

Patient A was a 20-year-old girl. The diagnosis was schizophrenic reaction, catatonic type. She presented a six-month history of progressive withdrawal, with depression of mood and a striking loss of interest in her surroundings. Unreality feelings were present and her affect was markedly diminished.

The metabolic studies in this patient are shown in Figure 1. During the control period, 2 interesting findings were revealed: (a) with a daily intake of 0.870 Gm. of calcium the calcium balance was negative, and (b) with a daily intake of 2.20 Gm. of phosphorus the patient was retaining 0.697 Gm. per day. This high degree of phosphorus retention persisted throughout the entire study.

In period 2, following the first intravenous injection of 500 micrograms (μg.) of triiodothyronine, there was a significant increase in urinary phosphorus. At the same time, the negative calcium balance was intensified. One week later, during period 3, a second intravenous dose of triiodothyronine (500 μg.) was administered. Immediately thereafter a significant increase in phosphaturia was again observed, but this time calcium balance shifted to retention, the change occurring primarily in fecal calcium. During the fourth metabolic period, triiodothyronine was instituted in daily oral doses of 100 μg., and in the fifth period the dosage was raised to 200 μg. daily. An associated rise in urinary phosphorus was seen initially, but it was quantitatively less and did not continue in spite of continued administration of the hormone. At the same time a markedly negative calcium balance was established, the loss occurring primarily in the stool.

Serum protein-bound iodine (PBI) studies and basal metabolic rates (BMR) were carried out during the study. The control level of PBI was 4.3–5.2 μg. per 100 ml.—well within normal limits. The BMR was initially depressed, ranging from -28 to -35 per cent, but there was no clinical evidence of hypothyroidism. During the administration of triiodothyronine, the PBI level was slightly depressed to 3.1 μg. per 100 ml., and the BMR rose to a maximum of $+12$ per cent.

Those psychologic changes which were striking and readily observable in this patient during the course of study are recorded in Figure 1, so that they may be compared with the metabolic alterations. Twenty-four hours after the first intravenous dose of triiodothyronine, during the second

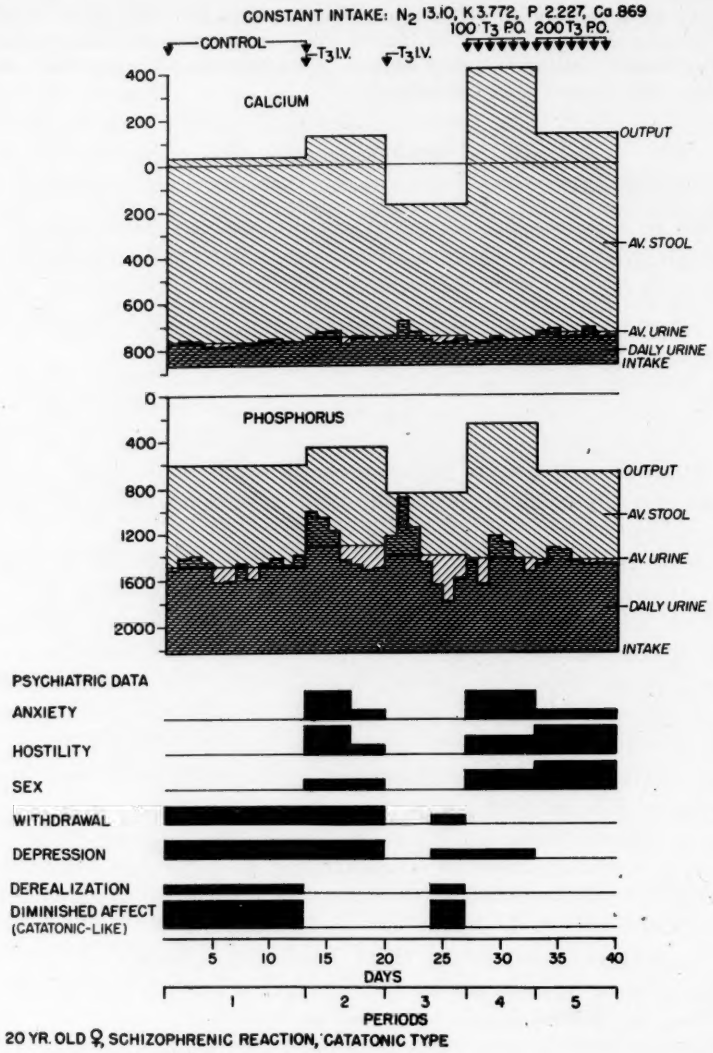

FIG. 1. Metabolic data—Patient A.

metabolic period, diminution of affect and loss of interest (which had been prominent features) were markedly altered, and the patient expressed intense anger and feelings of hopelessness. Tension and anxiety were prominent. Sexual features appeared in her social behavior. This state of strong emotions continued for four days. As the reaction subsided, she entered a

transient period of relative well-being, although some evidence of depression and withdrawal remained. Following the second intravenous dose, in period 3, the state of well-being continued for another four days, with a further decrease in withdrawal. This phase ended abruptly, however, and she returned to her original psychopathologic state. In period 4, while receiving 100 μg. daily of triiodothyronine, sexual behavior again became manifest, associated with marked anxiety and a moderate degree of hostility. When the dosage was increased to 200 μg. daily in period 5, hostility became intense; there were angry outbursts and paranoid misinterpretations of the environment. At the same time her dream-life became active, with well defined sexual content. Within twenty-four hours after the termination of triiodothyronine therapy the patient again began to show evidence of marked depression and withdrawal. She was mute, sullen, and without demonstrable affect. This state lasted for six days, when an abrupt clearing of her condition occurred. The subsequent improvement continued for many weeks. The peaks of overt emotion corresponded to the peaks of calcium loss.

Patient B (PWC-5)

Patient B was a 22-year-old girl. The diagnosis was schizophrenic reaction of undifferentiated type, with prominent psychoneurotic symptoms. She presented a four-month history of anxiety attacks, difficulty in concentration, marked introspection, hypochondriacal ruminations, loss of energy and feelings of unreality. Sexual interest had been completely absent since the onset of her illness.

Calcium and phosphorus studies are shown in Figure 2. Following con-

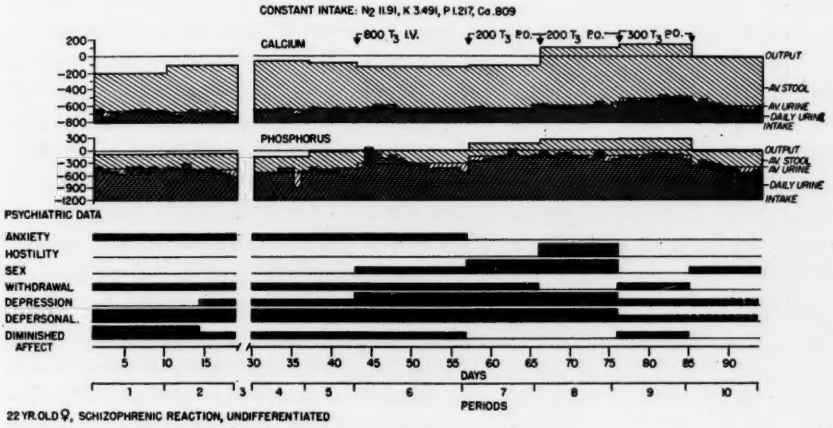

FIG. 2. Metabolic data—Patient B.

trol studies, a dose of 800 μg. of triiodothyronine was administered intravenously in period 6. Immediately thereafter a marked rise in urinary phosphorus occurred, but there was no essential change in calcium balance. After two weeks, during period 7, triiodothyronine was instituted in an oral dosage of 200 μg. daily. A gradual rise in urinary phosphorus was noted. No change in calcium balance occurred, however, until period 8, when it became negative. Subsequently, during period 9, the dosage of triiodothyronine was increased to 300 μg. daily. The negative calcium balance persisted, but there was no further increment in phosphaturia. A significant increase in urinary calcium was observed at this time. After the termination of triiodothyronine therapy, these changes were reversed, the negative calcium balance disappearing and the urinary loss of phosphorus returning to the pre-treatment level.

Serum protein-bound iodine and basal metabolic rate determinations were obtained. The control PBI level averaged 6.3 μg. per 100 ml., and no significant change was observed in this index during the administration of triiodothyronine, the values averaging 5.9 μg. per 100 ml. The BMR ranged from −12 to −22 per cent in the control period, and rose to a maximum of +8 per cent during triiodothyronine therapy. Radioactive iodine tracer studies were performed. The control thyroidal I^{131} uptake was 37 per cent, with a conversion ratio of 29 per cent—well within normal limits for this laboratory. It is of interest that while the patient was receiving 300 μg. of triiodothyronine daily, the thyroidal uptake was 20 per cent, indicating that the depression of activity was less than expected, *i.e.*, there was a higher degree of residual activity. During the two months following the termination of triiodothyronine therapy, the I^{131} uptake gradually returned to normal levels.

The psychologic changes which were observed in this patient during the course of study are included on Figure 2. During period 6, twenty-four hours after the initial intravenous injection of triiodothyronine, sexual manifestations appeared in her attitude toward the doctors and in her manner of dress. During period 7, when she was given 200 μg. of triiodothyronine daily by mouth, these manifestations were further intensified and sexual content appeared in her dreams. Concomitantly there was a slight decrease in anxiety, and increased contact and alertness. During period 8, while she was receiving 200 μg. of triiodothyronine daily, a moderate degree of hostility emerged. When the dosage was increased to 300 μg. daily in period 9, toxic symptoms of severe exhaustion and palpitation obscured underlying emotional changes. Following discontinuation of the drug, sexual unrest continued, but hostility diminished substantially. Depression of mood was reduced somewhat, and the unreality experiences began to decrease strikingly in intensity, frequency and duration. Gradual

symptomatic improvement continued during the ensuing weeks.

Here again, the period during which the patient experienced the most intense emotions corresponded to the period in which a negative calcium balance was established. In contrast to the findings in the preceding patient, a negative calcium balance did not appear until triiodothyronine had been given in higher dosages and for a longer period of time. Calcium loss occurred primarily by way of the stool.

Patient C (PWC-7)

Patient C was a 40-year-old premenopausal woman. The diagnosis was schizophrenic reaction of the paranoid type. There was a six-month history of severe depression of mood, paranoid delusions, and marked depersonalization: "my head is dried up, something vital is missing." Her affect was markedly diminished, except for resentment and associated suspiciousness, and she displayed little interest in her surroundings. These psychopathologic changes had taken place, however, against a background of twenty years of chronic withdrawal and personality maladjustment.

Metabolic studies are shown in Figure 3. During the control period, with a moderately high daily intake of 1.30 Gm. of calcium and 1.26 Gm. of phosphorus, the patient was in a state of negative calcium and phosphorus balance. In period 2, following the first intravenous injection of 500 μg. of triiodothyronine, a marked, but transient increase in urinary phosphorus occurred, in association with a slight shift in calcium balance in the direction of retention. One week later, during period 3, a second 500-μg. dose was given intravenously. At this time no change in calcium balance was observed. The increment in urinary phosphorus loss was extremely limited. During the fourth and fifth metabolic periods, the patient received 100 and 200 μg. of triiodothyronine daily by mouth. In these periods, a very slight increase in the loss of urinary phosphorus was noted. In contrast to the findings in the preceding patients, however, a strongly positive calcium balance was established.

Serum protein-bound iodine levels and basal metabolic rates were determined. The control PBI level was 5.5 μg. per 100 ml., and no change was noted during administration of triiodothyronine. The basal metabolic rate was −19 per cent in the control period, and reached a maximum of ±0 while the patient was receiving triiodothyronine.

The emotional and psychopathologic changes observed during the course of the studies are included in Figure 3. No emotional changes whatsoever were seen following the first injection of triiodothyronine. After the second intravenous dose, in period 3, there was a gradual diminution in hostility and related paranoid suspiciousness. Depersonalization was reduced and interest in her surroundings increased significantly. These changes were in-

tensified during periods 4 and 5, when triiodothyronine was administered orally. Mild sexual manifestations appeared while the patient was receiving 200 micrograms daily. Depression of mood, characterized by intense hopelessness, persisted unchanged throughout. Resentment and suspiciousness returned within forty-eight hours after the termination of therapy. Although this patient demonstrated more alertness and interest in her en-

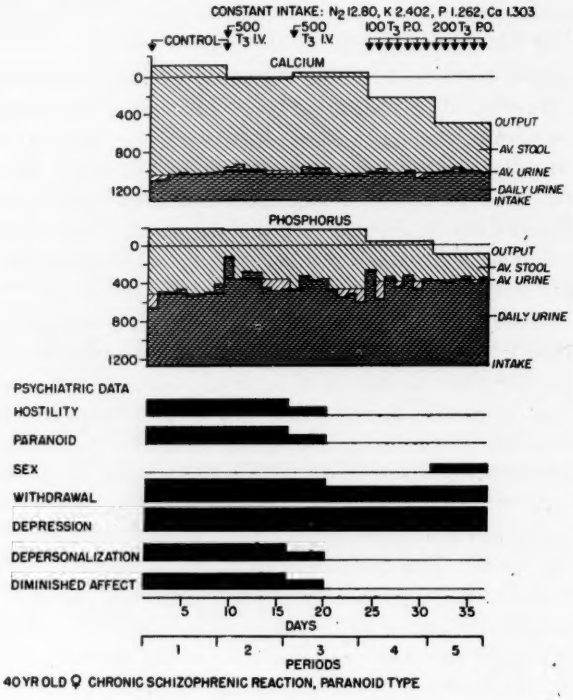

FIG. 3. Metabolic data—Patient C.

vironment while receiving triiodothyronine than previously, there was a gradual reduction in the intensity of pathologic emotions, particularly hostility, and this change was paralleled by a progressive, significant increase in calcium retention.

Comment

Figure 4 illustrates the effect of triiodothyronine on phosphorus metabolism in these 3 psychiatric patients, compared with the effect in Patient D who had myxedema. The data were analyzed to determine whether the loss of phosphorus during the administration of triiodothyronine corre-

sponded to, or exceeded that theoretically anticipated from the combined loss of nitrogen and calcium. These findings have been compared with those obtained in the studies of myxedema previously reported by Rawson *et al.* (13).

Patient A retained much more phosphorus during the control period than could be accounted for by the retention of nitrogen and calcium. The ad-

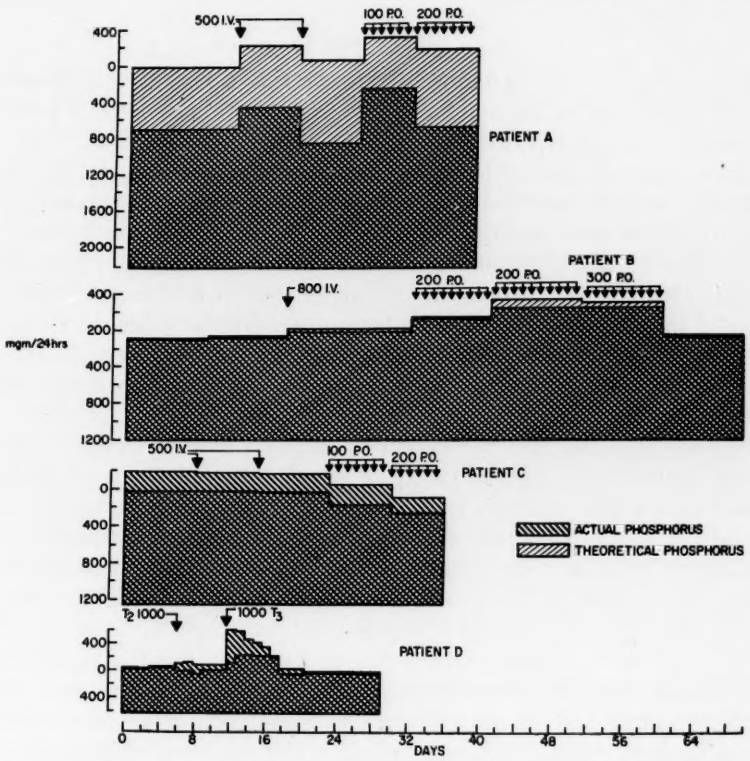

FIG. 4. Comparison of metabolic data in Patients A, B, C (schizophrenic) with those in Patient D (myxedematous).

ministration of triiodothyronine failed to correct this theoretically excessive phosphorus retention. Patient B retained approximately the amount of phosphorus expected on the basis of nitrogen and calcium retention throughout the control period. The administration of triiodothyronine again failed to produce an excessive loss of phosphorus; in fact, the phosphorus loss was slightly less than theoretically anticipated if calculated from calcium and nitrogen losses. In Patient C, an unusual relationship

between actual and theoretical changes in phosphorus was seen in the control period, namely, more phosphorus was lost than could be accounted for from nitrogen and calcium balances. Although the phosphorus balance became positive again during triiodothyronine therapy, the excessive loss (inadequate retention) of phosphorus persisted throughout. None of these patients, therefore, showed the excessive loss of phosphorus during treatment with triiodothyronine observed in myxedematous Patient D.

DISCUSSION

The failure of triiodothyronine to induce a loss of phosphorus exceeding that accounted for by the combined loss of nitrogen and calcium in these schizophrenic patients raises certain important considerations. This observation is at variance with the findings in myxedema (13). In the latter condition, some of the excess phosphorus may reflect the breakdown of phosphocreatine, and some may represent the ability of triiodothyronine to increase the rate of turnover in the adenosinetriphosphate system, as demonstrated by Klemperer (15). Although further studies to clarify the nature of this reponse are indicated, these observations appear to strengthen and enlarge those of Nagy and Gerty (16) and Gottlieb *et al.* (17), to the effect that in schizophrenic patients the response of phosphorylation reactions to insulin appears to be abnormal.

The metabolic patterns of calcium utilization observed in our 3 schizophrenic patients are representative of our findings in 10 other patients studied in a similar manner. In 5 of these 10 cases (4 schizophrenic reactions of the undifferentiated and simple type, 1 obsessive-compulsive psychoneurotic reaction) there was a calcium response to triiodothyronine similar to that seen in Patients A and B, namely, the loss of calcium was significantly increased, primarily due to fecal excretion. In these 5 patients the duration of psychiatric illness ranged from five months to six years, with a mean duration of twelve months. Prior to administration of the hormone, diminished emotional responsiveness, often associated with depersonalization, was a prominent finding. While receiving triiodothyronine, these patients experienced intense emotional reactions, the peaks of overt emotion corresponding in time to the appearance of negative calcium balances.

The remaining 5 cases (3 paranoid schizophrenic reactions, 1 undifferentiated schizophrenic state and 1 paranoid reaction) demonstrated calcium responses to triiodothyronine resembling those seen in Patient C, namely, calcium balance was either unaffected or shifted in the direction of increased retention. The duration of psychiatric illness in this group ranged from three to sixteen years, with a mean duration of six years. Intense emotional reactions were not apparent in these patients during

the administration of triiodothyronine; in fact, reduction in overt hostile emotions was apparent in 2 cases.

It is not clear what factors determined whether or not there would be a pronounced loss of calcium during administration of triiodothyronine in these patients. In the latter group there appeared to be a trend toward longer duration of illness and paranoid subgrouping.

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FUNCTIONAL RELATIONSHIPS BETWEEN THE LIVER, THE THYROXINE-BINDING PROTEIN OF SERUM, AND THE THYROID*

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ABSTRACT

Various aspects of the effect of liver damage on thyroid function were studied in 35 cases of liver disease (with 30 normal controls) and in a group of thyroidectomized rats made toxic with carbon tetrachloride. Acute, severe, diffuse liver damage (as in infectious hepatitis) temporarily provokes a statistically significant increase in the level of serum protein-bound iodine (PBI), associated with a higher rate of fixation of thyroxine to the serum thyroxine-binding protein (TBP). These abnormal findings are caused by different mechanisms, such as disturbance of biliary thyroxine excretion, and lack of deiodination and glucuroconjugation of thyroxine in the damaged liver cell. Under these conditions, the thyroxine turnover is slower and thyroxine accumulates in the circulation. The increased serum PBI level is not associated with hyperthyroidism, although the content of the PBI in infectious hepatitis is practically built from chromatographically demonstrable pure thyroxine. The data do not confirm the hypothesis of a pathologic binding of thyroxine to plasma protein, but the high TBP level in hepatitis could contribute to the non-utilization of thyroxine in the tissues. On the other hand, experiments in rats show that the damaged liver cell is also the cause of the lack of metabolic response in this particular type of hyperthyroxinemia.

INTRODUCTION

IN RECENT years much experimental and clinical work has demonstrated the important part played by the liver in the regulation of the catabolism of the thyroid hormones. In fact, as Gross and Leblond showed in 1947 (1), thyroxine is not only selectively taken up by the liver, but actually penetrates into the hepatic cells (2). It is then secreted through the bile ducts, partially in the form of glucuro-conjugates (3, 4) and α-keto-derivatives (5, 6). Some appears in the stools, and another part is reabsorbed by the intestinal wall, thus establishing an enterohepatic circulation (7–9). Thyroxine can also be deiodinated in the liver (10–14).

Therefore, it is clear that the liver plays an important role in the regulation of the blood level of the thyroid hormones. Certain clinical observa-

Received August 1, 1958.

* Presented at the Annual Meeting of the American Goiter Association, San Francisco, California, June 17–19, 1958.

tions suggest that the liver is directly involved in some disorders of thyroid function. Liver damage and, in certain instances, cirrhosis are observed in cases of severe hyperthyroidism.

In 1951, Kydd and Man (15) found that the serum protein-bound iodine (PBI) level was elevated in infectious hepatitis and also often in liver cirrhosis. Confirming this observation, the present report describes the alteration of thyroid function provoked by diffuse liver damage and discusses the functional relationship between the liver, the intermediary thyroxine metabolism and the action of the specific thyroxine-binding protein (TBP).

METHODS

Serum PBI concentration was measured by the method of Barker *et al.* (16), adapted for micro-quantities of 200 microliters of serum. Each estimation was performed in duplicate. For the tests with radioiodine, the patient received 100–200 μc. as potassium iodide. a) The fixation in the thyroid was estimated by means of a scintillation counter. Activity is expressed as a percentage of the dose ingested (17). b) The conversion index formulated by Sheline, Clark and associates (18, 19) was used for calculation of the percentage of radioactivity bound by protein and the intensity and rapidity of the hormonal secretion.

Chromatograms were developed from aliquots of blood, bile, urine and tissue homogenates, after acidification to pH 2.5 and extraction by butanol saturated with thiosulfate. The butanol extracts were concentrated *in vacuo* under nitrogen before being placed on the chromatograms. The solvents usually employed were butanol saturated with 2*N* ammonia (descending method), and collidine/H_2O (ascending method) (20, 21).

The turnover of thyroxine (chromatographically pure, and tagged with I^{131}) was studied according to the technique of Ingbar and Freinkel (22). The plasma activity found five minutes after injection was considered as the starting value.

The test for saturation of TBP by thyroxine was performed by the method of Freinkel and others (23–25).

In the rat, measurement of oxygen consumption was carried out by means of the apparatus of Bargeton and Krum-Heller (26). Rats, weighing about 200 Gm., and thyroidectomized three weeks previously, were used. They were fasted for five to eight hours before the determination—which is valueless if the rat is not asleep (without anesthesia). Each measurement was the mean of 6 readings made every five minutes. Thyroxine (Roche) was injected intravenously in a dosage of 2 gamma of iodine per 100 Gm. of body weight. In the studies on thyroxine bound to the fractions of Cohn (fractions IV 6 and fraction V), the dose of protein injected was 40 mg. per 100 Gm.of body weight, the fractions having been incubated for thirty minutes at 37° C. with thyroxine just before injection. The oxygen consumption was measured before injection, then 12, 18, 24, 36 and 48 hours, and 4, 7 and 10 days after injection. The normal oxygen consumption of thyroidectomized rats is 831 ± 37 cc. per Kg. per hour (mean for 56 rats).

RESULTS

Thyroid fixation, conversion index and serum PBI level in liver disease

The results are summarized in Figure 1. The uptake of I^{131} by the thyroid and the conversion index in 19 cases of infectious hepatitis were com-

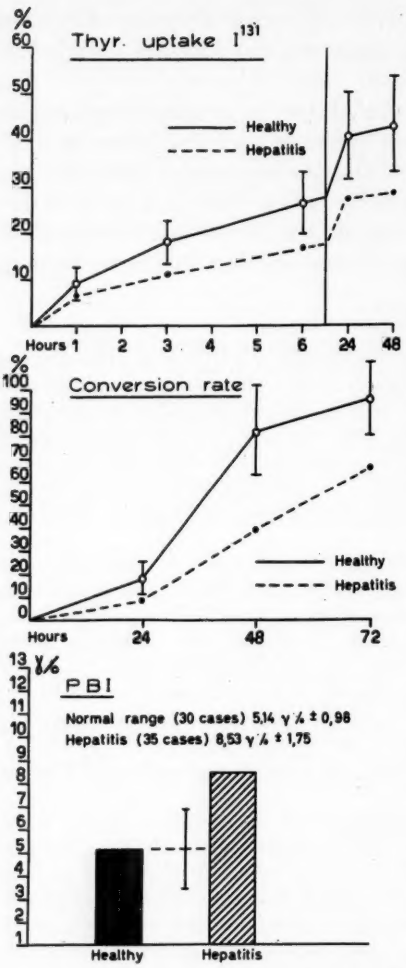


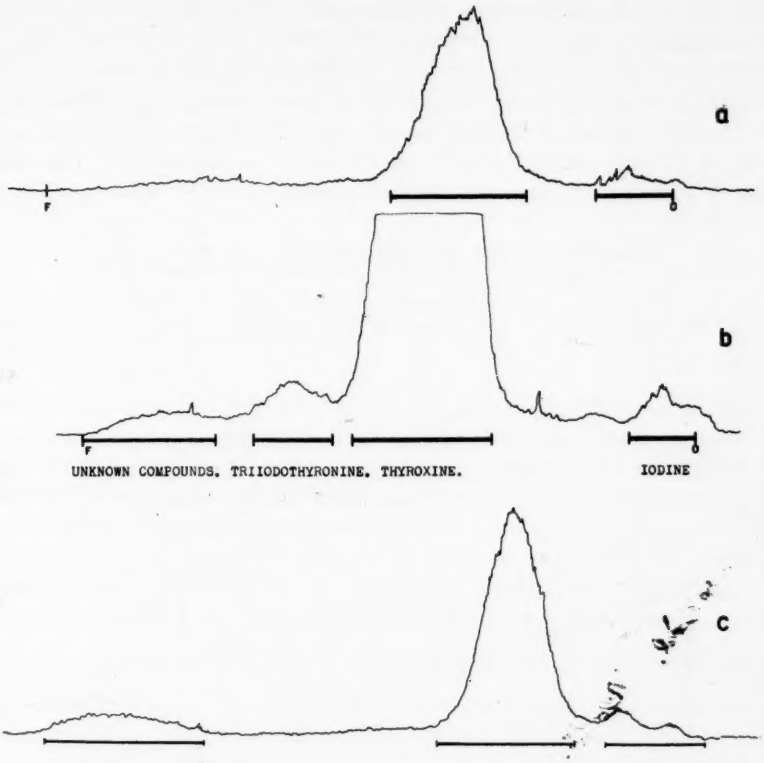
FIG. 1. Thyroidal uptake of I^{131} and conversion rate in 19 cases of hepatitis compared with data in 30 normal subjects; and serum PBI level in 35 cases of hepatitis compared with that in 30 healthy people. Mean: $\pm\epsilon$ 0.01 ("Student" test (42)).

pared with those in 30 normal subjects; the PBI level in 35 cases of hepatitis was also compared with that in 30 normal subjects. In infectious hepatitis the PBI level was statistically increased although thyroidal activity remained low. In hepatic cirrhosis analogous results were obtained, although statistically not significant. Thus the conclusion could be drawn that the high level of protein-bound iodine in cases of diffuse liver damage is not due to hyperactivity of the thyroid.

To demonstrate in which form this increased PBI circulated, chromatograms were run of butanol extracts of sera from normal people and patients with infectious hepatitis or obstructive jaundice, twenty-four hours after oral administration of I^{131}. These were compared with chromatograms of bile (method of Roche (5)). In the normal subjects the serum contained only free thyroxine and iodine-I^{131}, whereas bile contained free and glucuroconjugated thyroxine (27). In infectious hepatitis the serum and the bile contained only free thyroxine or, in rare cases, traces of glucuronic acid compounds in the bile. In cases of obstructive jaundice, glucuroconjugates were found regularly in serum, thus confirming in human beings the observations of Roche on animals with ligated bile ducts (5). These observations lead to the conclusion that normally in human beings, the liver eliminates thyroxine both in the free form and in the form of conjugated compounds with glucuronic acid, and that obstruction of the bile duct provokes an accumulation of these glucuroconjugates in the

blood. In infectious hepatitis the liver cell seems to lose the capacity for conjugation of thyroxine.

In order to confirm this hypothesis experimentally we studied *in vitro* the deiodination of thyroxine in rat liver slices (28), and *in vivo* the glucuroconjugation of thyroxine in rat liver after injection of chromatographically pure thyroxine (29). The incubation technique of Albright (30) was used. It was found that the liver of normal rats can deiodinate thyroxine to triiodothyronine (Fig. 2) and transform thyroxine into its glucuroconju-



Incubation of labelled thyroxine with rat liver slices.
a) Time O.
b) After 15 minutes of incubation.
c) With liver slices of rats intoxicated with allyl formiate, after 15 minutes of incubation.

FIG. 2. Chromatograms of rat liver. Solvent: butanol-NH_3/2*N*; Whatman No. 1 paper. Chromatograms were registered by a Geiger counter connected with an integrator. O: origin of chromatograms. F: front of solvent.

gate. However, when liver slices from rats poisoned with allyl-formate were incubated, the deiodination of thyroxine was greatly decreased and the glucuroconjugation of thyroxine was stopped.

Turnover of radiothyroxine in liver disease

The foregoing observations indicated that the increased PBI level in infectious hepatitis corresponds to an inhibition of the deiodination of thyroxine and of its glucuroconjugation. Thus in diffuse liver damage the turnover of thyroid hormone is slowed. In fact, as shown in a previous study (31) involving the effect of intravenous injection of 5–10 gamma of chromatographically pure thyroxine-I^{131} (300 microcuries) into normal subjects and patients suffering from infectious hepatitis, the rate of the decrease of radioactivity in the plasma is much slower in infectious hepatitis. As did Sterling *et al.* (32), in the normal subject we observed a decrease of 50 per cent in activity on the sixth to seventh day (6.2 days, mean of 3 cases), whereas we noted the same decrease of activity in the

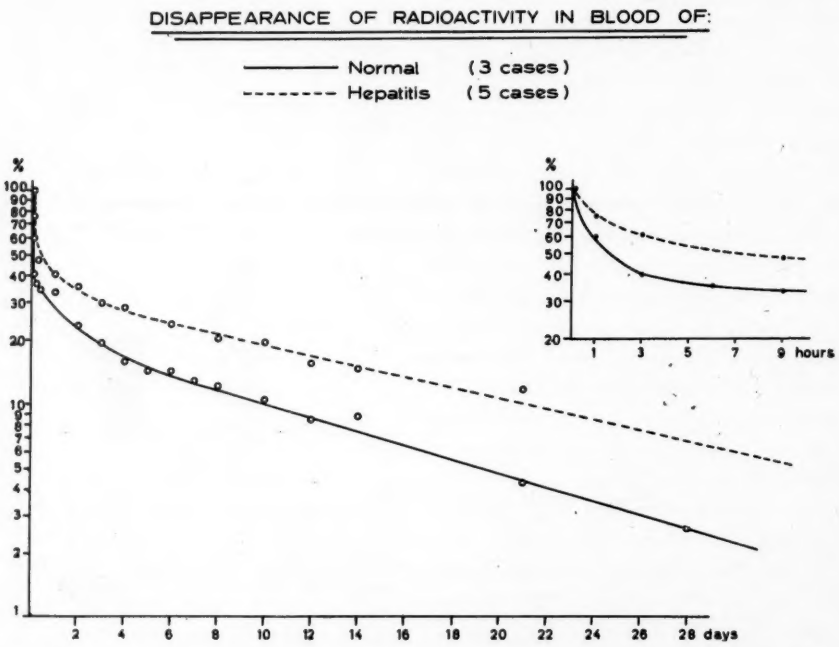


FIG. 3. The activity in plasma five minutes after the injection of thyroxine-I^{131} chosen as a 100% level; all the measurements are referred to this one and plotted on logarithm paper. The turnover rate is calculated from the value on this graph between 2 days and 12 days.

hepatitis cases on the ninth to tenth day (9.3 days, mean of 5 cases) (Fig. 3). By chromatography of the butanol extract of the serum, we found that the concentration of thyroxine-I^{131} ten days after injection was higher in a patient suffering from hepatitis than in a normal subject (Fig. 4).

Intestinal elimination of I^{131} was lower in patients with infectious hepatitis than in normal subjects, due to a slowing down of the enterohepatic circulation, but urinary elimination was normal, as shown in the following data:

ELIMINATION OF I^{131} AFTER INJECTION OF RADIOTHYROXINE (EXPRESSED AS PERCENTAGE OF THE DOSE)

	Normal subjects (3 cases)	Hepatitis patients (5 cases)
In urine		
24 hrs. after dose	17.5%	13.4%
48 hrs. after dose	22.0	17.9
7 days after dose	34.8	31.7
21 days after dose	47.5	41.4
In feces		
24 hrs. after dose	2.5	0.9
48 hrs. after dose	4.1	1.0
7 days after dose	8.3	3.3
14 days after dose	12.1	5.7

The catabolism and the excretion of thyroxine are slackened in cases of diffuse liver damage, resulting in an increase of circulating thyroid hormone. This finding raises a new important problem—Why is the basal metabolic rate not increased in infectious hepatitis and in all of the cases of severe, diffuse liver damage in which the thyroxine level in the blood is elevated? The situation resembles that in pregnancy (33, 34) or during treatment with estrogens (35–37). Similarly, the hyperthyroxinemia of infectious hepatitis is not followed by symptoms of hyperthyroidism (38).

Thyroxine-binding protein (TBP) saturation test of Freinkel in liver disease

In order to study the relationship between thyroxine and its specific serum protein carrier, we used the TBP saturation test of Freinkel.

Studying the serum of normal people and of patients with infectious hepatitis by means of this test (23), we constantly observed a higher fixation rate of thyroxine in hepatitis serum as compared with normal serum; this difference was statistically consistent. With a PBI level of 40 gamma per 100 ml., 45 per cent of the thyroxine radioactivity was fixed by the TBP in normal serum, but to obtain the same fixation rate in hepatitis serum it was practically necessary to have a thyroxine level of 80 gamma per 100 ml. (Fig. 5) (39).

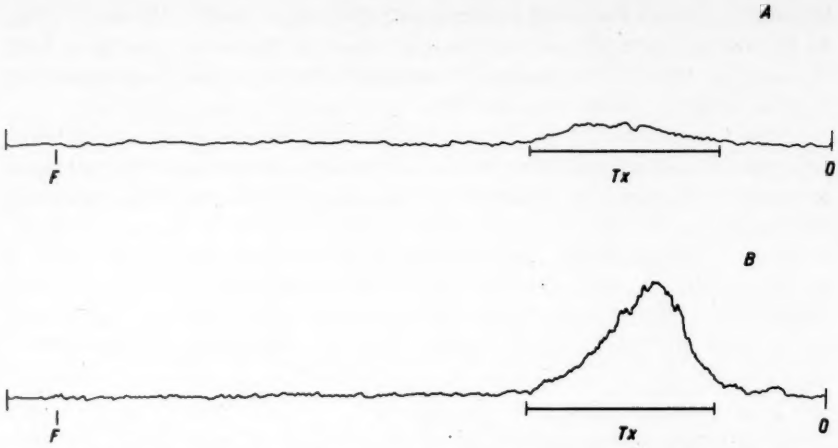


FIG. 4. Chromatograms of blood from: A. Normal subject, ten days after injection of radiothyroxine. B. Hepatitis patient, ten days after injection of radiothyroxine. (Solvent: butanol-NH_3/2*N*.)

These results indicate either that, in infectious hepatitis, the TBP is capable of binding more thyroxine than is the TBP of normal persons, or, that the level of TBP is higher in hepatitis. It is possible and probable that in hepatitis the TBP level is higher (but we were not able to demonstrate it). If this be so, it would seem that in this disease, as in pregnancy, the TBP binds the circulating thyroxine and thus decreases the amount of thyroxine available for the tissues. It is possible that the binding of thyroxine to its protein could, in infectious hepatitis, inactivate the hormone. Though this hypothesis is less probable and not physiologic, we tried to study in thyroidectomized rats the metabolic effect of injection of free thyroxine, and of thyroxine incubated with human serum albumin and with fraction IV 6 of Cohn extracted from normal and hepatitis serum (40, 41). This fraction contains the TBP (23). The control animals received injections of normal TBP and hepatitis TBP without thyroxine.

Rate of oxygen consumption in normal rats and in rats made toxic with carbon tetrachloride

The results of these experiments are shown in Figures 6 and 7. The metabolic response in the first control group receiving an injection of thyroxine alone (12 rats) was compared with the metabolic response to the same dose of thyroxine previously incubated with TBP of normal human beings (8 rats), and with the response in a group of 6 animals receiving thyroxine in-

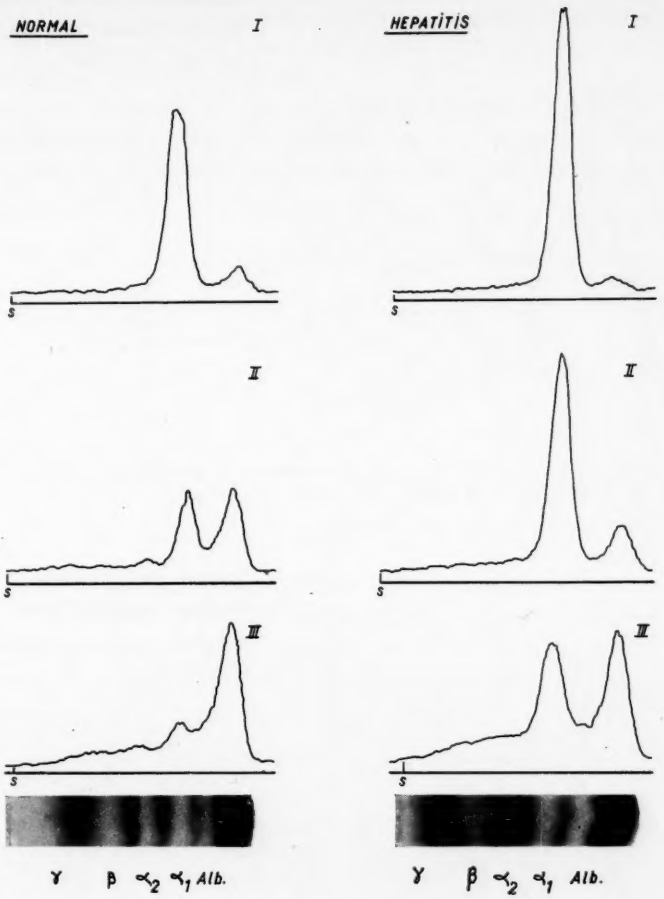


FIG. 5. Saturation test. Electrophoresis of normal and hepatitis sera incubated with ratiothyroxine; PBI adjusted to: I, 10 γ; II, 40 γ; and III, 80 γ per 100 ml. S = origin of electrophoresis. Electrophoresis patterns scanned with a Geiger counter before staining with Black Sudan. Veronal buffer pH 8.4, ionic strength, 0.1.

cubated with TBP from a case of infectious hepatitis. The effect of the injection of TBP alone (without thyroxine) was also studied (Fig. 6).

In this series of experiments there was no statistically significant difference between the responses to thyroxine, whether the thyroxine was free or bound to normal TBP. An increase of oxygen consumption twelve and eighteen hours after the injection of thyroxine incubated with hepatitis

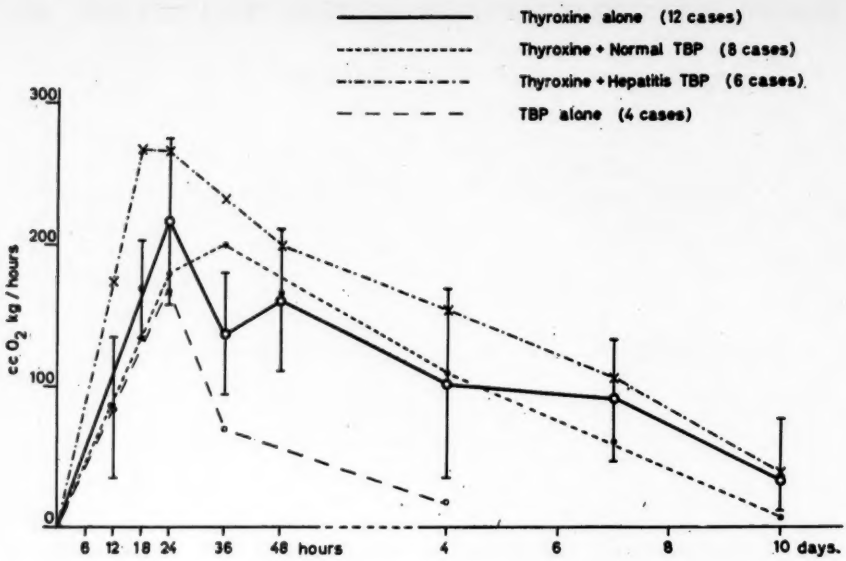

Thyroxine alone (12 cases)
Thyroxine + Normal TBP (8 cases)
Thyroxine + Hepatitis TBP (6 cases)
TBP alone (4 cases)
300
200
100
0
cc O_2 kg / hours
6 12 18 24 36 48 hours 4 6 8 10 days.

FIG. 6

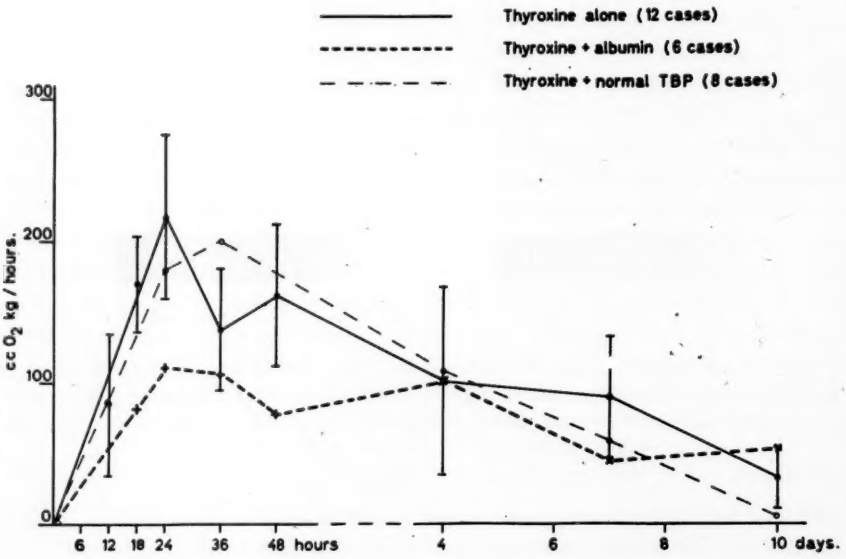

Thyroxine alone (12 cases)
Thyroxine + albumin (6 cases)
Thyroxine + normal TBP (8 cases)
300
200
100
0
cc O_2 kg / hours.
6 12 18 24 36 48 hours 4 6 8 10 days.

FIG. 7

FIGS. 6 and 7. Augmentation of oxygen consumption in thyroidectomized rats after injection of thyroxine and TBP, alone and in various combinations. Capped perpendicular lines = standard deviation.

$$\epsilon 0.01 = \sqrt{\frac{\Sigma x_i^2 - m\Sigma x_i}{(N-1)}} \times \frac{1}{N} \times 2.58$$

TBP was observed. This was probably due to the high thyroxine content of the TBP fraction added to the thyroxine. TBP alone produced only a small and short metabolic activation.

Thus that the incubation of the thyroxine with human TBP did not reduce the physiologic effect of thyroxine on metabolism. The thyroxine-TBP complex from normal human serum and from infectious hepatitis

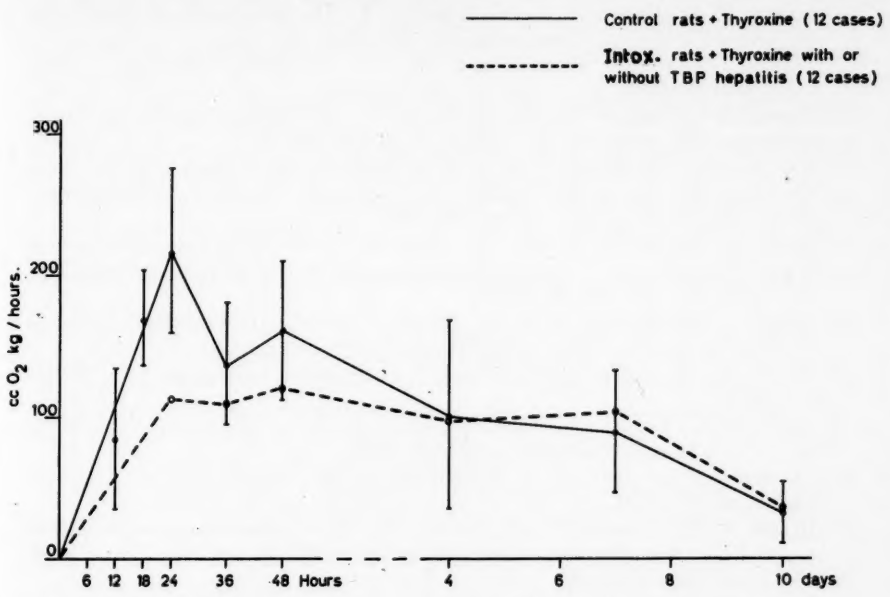


FIG. 8. Diminution of oxygen consumption in CCl_4-intoxicated rats after injection of thyroxine with or without hepatitis TBP. (Rats were made toxic with 0.4 ml. of a 20 per cent solution of carbon tetrachloride in olive oil per day per 100 Gm. during ten days.)

serum had a normal stimulating metabolic action in these thyroidectomized animals. It appears that the thyroxine-TBP complex is rapidly destroyed in rats and that the thyroxine so liberated can provoke the observed metabolic effect. Against this hypothesis is the fact that the thyroxine-albumin complex had only a slight metabolic effect under the same experimental conditions (Fig. 7).

In a final group of experiments, we studied (under the same conditions) the effect of liver damage on the action of thyroxine. In 12 thyroidectomized rats the liver damage due to carbon tetrachloride poisoning produced a statistically consistent inhibition of the effect of thyroxine and of thyroxine+TBP on oxygen consumption (Fig. 8).

The normal metabolic response of the body to the action of thyroxine seems to be related to the intact function of the liver cell.

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PSEUDO-PSEUDOHYPOPARATHYROIDISM WITH UNUSUAL FEATURES

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ABSTRACT

A case of pseudo-pseudohypoparathyroidism with some unusual features is presented. The patient, a 64-year-old diabetic female, was short, somewhat obese, and had a shortened metacarpal of the right ring-finger. The only subcutaneous calcification or ossification was a hitherto undescribed finding in this disorder, namely, ossification of the pinna of each ear. Plasma calcium and phosphorus concentrations were normal. Injection of parathyroid extract of proven potency (Ellsworth-Howard test) did not produce phosphate diuresis, but did increase plasma calcium concentration. Intravenous calcium infusion caused the expected increase in urine and plasma calcium content, but was not followed by the expected normal decrease in urinary phosphate excretion. These findings suggested that there might be a defect in the control of tubular reabsorption of phosphate by parathyroid hormone in this patient, whereas the action of the hormone on calcium metabolism was normal.

THE term pseudo-pseudohypoparathyroidism was coined by Fuller Albright in 1952 to describe a young woman who had a round face, short stout build, short metacarpals, apparently normal calcium metabolism, and normal parathyroid function (1). He used this term because the patient resembled a larger group (2, 3) with similar physical characteristics who had hypocalcemia, hyperphosphatemia, and subcutaneous calcification and did not respond to intravenous parathyroid extract (P.T.E.) with phosphate diuresis (Ellsworth-Howard test) (4). Albright suggested that this latter group of patients demonstrated a state of end-organ failure, that is, failure of the kidney to respond to parathyroid hormone. He called the condition pseudo-hypoparathyroidism because of the chemical similarity to, but etiologic difference from idiopathic hypoparathyroidism (2). The 1952 case of pseudo-pseudohypoparathyroidism demonstrated the skeletal abnormalities in association with normal levels of serum calcium and phosphorus. This seemed to bear out Albright's thesis that the dyschondroplasia, subcutaneous calcifications and renal insensitivity to P.T.E. could be related genetically, but not chemically, since they could occur independently of each other. Since this report several other cases

Received November 5, 1958.

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which fit this description have been documented (5–11). The present case may represent another example of this disorder. However, in this patient the metabolic data suggest an abnormality of the renal response to parathyroid hormone.

CASE REPORT

A.K., a 65-year-old white widow of Polish extraction, was admitted to the Syracuse University Hospital on April 8, 1958, for study. Because of language difficulty, it was not possible to obtain a satisfactory history of her early life. She had 3 stillbirths at term. Her only living child was delivered by cesarean section in 1921. At the age of 42 years, she had undergone a supracervical hysterectomy and bilateral salpingo-oophorectomy for a right ovarian cyst (benign). Her menstrual cycles had been normal until their termination by this procedure. Diabetes mellitus was discovered at age 54. It was easily controlled with a 1500 calorie diabetic diet and 25 units of protamine zinc insulin daily. In 1950 she was admitted to Syracuse University Hospital for an ankle injury and at that time it was noted that her ears were "stony hard" and that her right ring-finger was shorter than normal. The serum calcium level was 12.2 mg. per 100 ml., phosphorus 3.8 mg. per 100 ml. and alkaline phosphatase 1.0 Bodansky unit. In August 1957 the patient was admitted to the Gynecological Service at Syracuse Memorial Hospital because of dysuria and frequency of urination. A cystocele and urethrocele were repaired and the cervix was removed. Postoperatively she became confused and fell out of bed, sustaining an intertrochanteric fracture of the left hip. This was pinned and healed satisfactorily.

There was no family history of diabetes, skeletal abnormalities, or consanguinity.

Physical examination revealed a short, thick-set, round-faced woman who weighed 120½ pounds and was 4 feet 8 inches in height. The mental status was difficult to determine because of the language barrier. The blood pressure was 180/90 mm. Hg, pulse 80, respirations 20 and temperature 100° F. Early bilateral cataracts were noted. Both external ears were stony hard and immobile. Her hands and feet were short, but the length of the digits appeared to be in proportion to her stature except for the ring-finger on the right. This finger was of the same length as the little finger on that hand; and on clenching the fist, it was evident that the knuckle of the ring-finger was absent (Fig. 1). The finger nails were broad and short. There was pain and limitation of movement of the left hip joint. Results of neurologic examination (including Chvostek and Trousseau tests) were normal.

Routine laboratory findings were normal. The urine (S.G. 1.022) contained no albumin or sugar and only a rare white blood cell per high-power field. The serum nonprotein nitrogen level was 40 mg. per 100 ml. and creatinine clearance was 81 cc. per minute. Serum total protein concentration was 6.7 Gm., albumin 4.3 Gm., and globulin 2.4 Gm. per 100 ml.; the fasting blood sugar level was 111 mg. per 100 ml. and the two-hour postprandial blood sugar level was 139 mg. per 100 ml. A urine culture produced no organisms. X-ray examination of the ears showed definite bone formation (Fig. 2) in both pinnae, and x-ray examination of the hands showed marked shortening of the metacarpal of the right ring-finger and slight shortening of the metacarpal of the left ring-finger (Fig. 3). X-ray films of the skull showed no calcification of the basal ganglia. The lamina dura of the teeth was intact, and there were no corneal calcifications by slit-lamp examination.

Special studies. While the patient was receiving a 1300-calorie daily diet containing 200 mg. of calcium, the plasma calcium level was 10.0 mg. per 100 ml., phosphorus 3.5

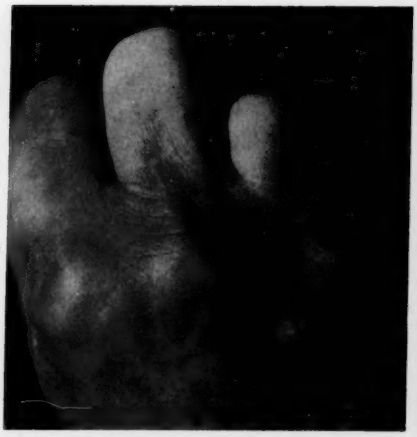

FIG. 1

FIG. 2

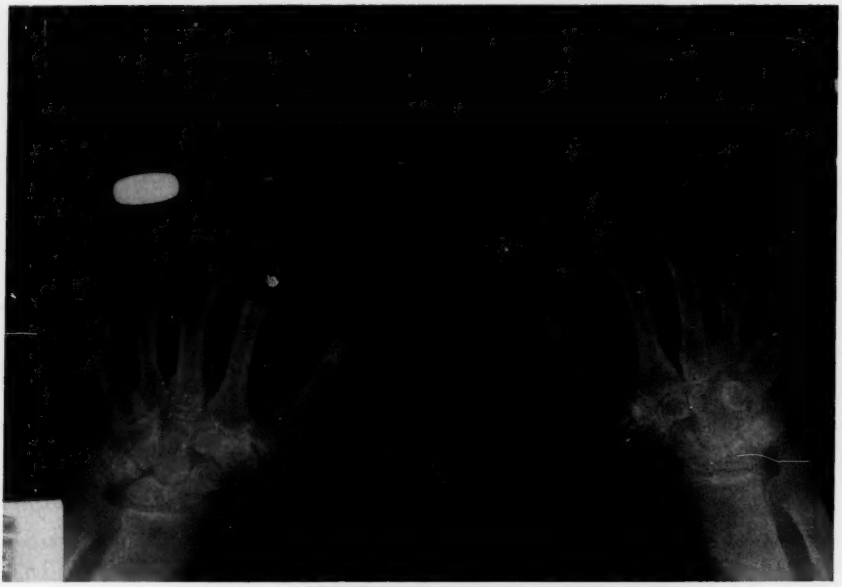

FIG. 3

FIG. 1. Right hand, showing absence of knuckle of the ring-finger.

FIG. 2. Roentgenogram showing bone formation in the aural pinna.

FIG. 3. Roentgenogram of both hands showing pronounced shortening of the metacarpal of the right ring-finger and slight shortening of the metacarpal of the left ring-finger.

mg. per 100 ml. (12, 13), and alkaline phosphatase 2.8 Bodansky units. An Ellsworth-Howard test was performed (4) using 200 units of parathyroid extract (Lilly, Lot #2095-687712) (Fig. 4). This extract caused phosphate diuresis in a normal subject but did not increase phosphate excretion in this case. It did, however, cause a rise in the concentration of plasma calcium from 10.0 mg. to 14.8 mg. per 100 ml. eighteen hours after its administration. The effect of an infusion of 15 mg. of calcium per Kg. of body weight, given as the gluconate, was observed and compared with the effect on the preceding

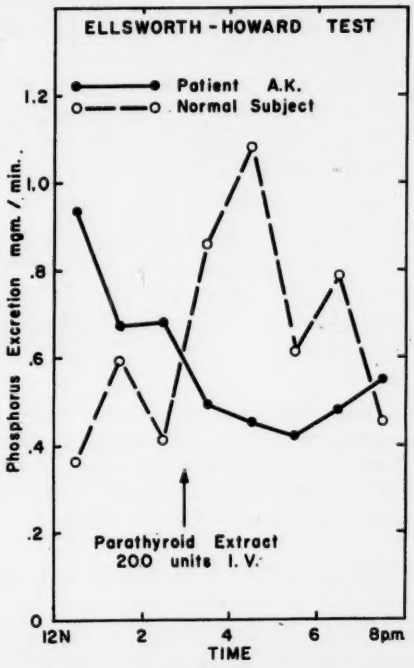

FIG. 4. Ellsworth-Howard test in Patient A. K. *vs.* normal subject. Parathyroid extract (200 units) was injected intravenously at 3 p.m. There was no phosphate diuresis in Patient A. K. but marked phosphate diuresis in the normal control.

control day (14) (Fig. 5). This infusion did not cause a decrease in phosphate clearance when compared with the values on the control day, but did cause an increase in the plasma levels of calcium and phosphorus. The calcium excretion on the day of infusion minus the average calcium excretion on the control day represented 47 per cent of the injected calcium load. Thus approximately 53 per cent was retained, which is a normal value. When the ratios of phosphate clearance to creatinine clearance were plotted to account for possible emptying errors, there was essentially no change in the results.

DISCUSSION

This patient presented a short, thick-set build, round facies, ossification of the ears, lenticular opacities, a shortened metacarpal of the right ring-finger, and normal plasma calcium and phosphorus levels. She thus fulfilled the criteria for a patient with pseudo-pseudohypoparathyroidism.

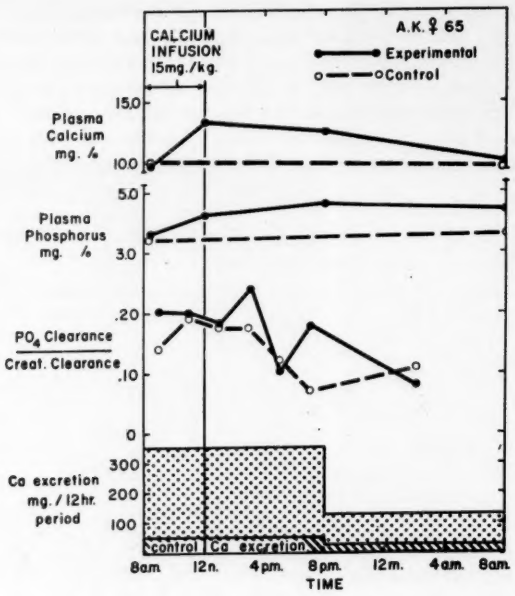

FIG. 5. Calcium infusion test in Patient A. K. An infusion of 820 mg. of calcium (as gluconate) in 100 ml. of 5 per cent dextrose in water was given between 8:00 a.m. and 12 noon. Compared with the data of the control day, there was a rise in the concentration of plasma calcium and phosphorus, but little change in the ratio of phosphate clearance to creatinine clearance. Calcium excretion increased markedly.

Calcification of the ears is an unusual finding and has not been previously reported in patients with pseudo- or with pseudo-pseudohypoparathyroidism. Calcified pinnae have been observed in cases of trauma to the ears, frostbite, Addison's disease (15), acromegaly (16) and ochronosis (17).

Of particular interest in the present patient are the results of the Ellsworth-Howard test and of the calcium loading test. A review of 5 previous Ellsworth-Howard tests performed in this syndrome (1, 5–8) revealed that in 3 instances the response of phosphate excretion to injection of parathyroid extract was either slight or nil (1, 7, 8). This was explained in 2 cases as being due to impotent extract (1, 8). However, Wallach, Englert and Brown (7) reported a patient in whom there was only a slight response to an extract that was shown to be potent in a control subject. In our patient there was no response, yet the extract was potent in a normal subject. Furthermore the extract caused an increase in the patient's plasma calcium level measured eighteen hours after injection. This finding suggests the possibility that her kidneys were unresponsive to parathyroid extract, but that the skeleton was responsive. This hypothesis was to some

extent confirmed by the results of the calcium loading test. With calcium loading, one can expect a decrease in phosphate clearance, ascribed to the increased plasma calcium concentration. The absence of a fall in phosphate clearance observed in this case suggests that if parathyroid hormone had indeed been "shut off" by the calcium infusion, the result was not a decrease in phosphate excretion; in fact, phosphate excretion increased. Further studies would be required to make certain that the patient had an abnormality in the renal handling of phosphate. Even then it could not be established that it was a congenital defect, since it might be related to acquired renal tubular disease in this elderly diabetic. Hiatt and Thompson showed that phosphaturia in the Ellsworth-Howard test may be entirely due to a rise in the glomerular filtration rate (18). Another equally speculative possibility is that the defect in this patient involved a diminished secretion of only part of the parathyroid hormone complex. Some authors have suggested (19) that parathyroid secretion can be divided into 2 moities, one acting chiefly on bone to raise the plasma calcium level, and the other acting chiefly on the kidney to increase the excretion of phosphate. Studies on the patient described in the present report suggest that she represents an intermediate syndrome between cases with congenital skeletal abnormalities (termed pseudo-pseudohypoparathyroidism) and those with additional abnormalities of calcium and phosphorus metabolism (termed pseudo-hypoparathyroidism).

Acknowledgment

The authors wish to acknowledge the technical assistance of Miss Patricia Maley.

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Letters to the Editor

A EUTHYROID MAN WITHOUT THYROXINE-BINDING GLOBULIN*

To the Editor:

It would seem that the thyroxine-binding globulin in blood should have a significant role in the transportation and the control of the free thyroxine available for the peripheral tissues (1–7). After the performance of several hundred determinations of thyroxine-binding globulin (TBG) (8) in hospital patients and industrial workers on the job (9), we are not clear as to the physiologic effect, if any, of the blood thyroxine concentration on the amount of TBG, or of the latter on the former; and not at all clear that the PBI/TBG relationship controls the amount of free thyroxine presented to the tissues. Recently we tested a man in an industrial survey; he was found to be euthyroid and without TBG.

W.W. was a 38-year-old, white, hard-working business executive who was normally developed, well nourished, and had no systemic complaints. No siblings or other relatives were available for study.

His medical history included a serious attack of infectious hepatitis in North Africa five years previously. At the time of the present examination the liver was not enlarged and there was no evidence of either cirrhosis or bile retention, but the cephalin flocculation reading was 2 plus in twenty-four hours and thymol turbidity was read as 2 units. The findings on physical examination were noncontributory. There was no thyroid enlargement.

The BMR was −9 per cent, pulse 66, serum protein-bound iodine level 3.0 micrograms per 100 ml. (average), and serum cholesterol level 308 milligrams per 100 ml. Results of complete blood counts and urine examinations were normal. The concentration of total serum protein was 7.3 grams per cent and the various fractions were normal by electrophoresis.

Several determinations of the binding of radioactive thyroxine *in vitro*, by the method of Robbins and Rall, indicated absence of TBG (Fig. 1). The same procedure carried out with radioactive tetraiodothyropropionic

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* Presented in part at the Annual Meeting of the American Goiter Association, San Francisco, California, June 17–19, 1958.

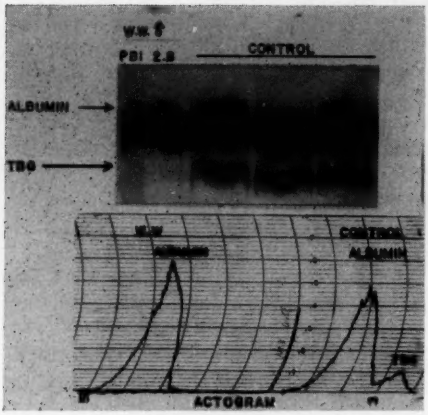

FIG. 1. Electrophoresis of sera containing 105 micrograms of *l*-thyroxine per 100 ml., in the subject without TBG and in a normal control.

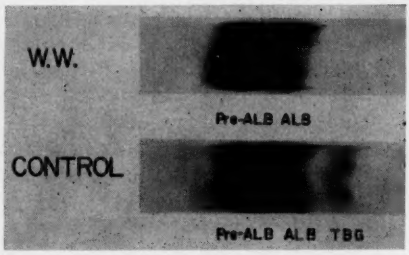

FIG. 2. The binding of tetraiodothyropropionic acid by serum proteins in Subject W. W. and in a normal control.
Pre-ALB = Pre-albumin
ALB = Albumin
TBG = Thyroxine-binding globulin.

acid also indicated the absence of TBG (Fig. 2). As may be seen, the deaminated thyroxine migrated with albumin and pre-albumin serum components, but in the sample of Patient W.W.'s serum it failed to localize in the region of the thyroxine-binding globulin.

In this man sufficient free thyroxine was reaching the tissues to maintain normal metabolism. It was being transported there either in combination with albumin, or free in the plasma, or in both forms. This case serves to illustrate that the thyroxine-binding serum globulin is not necessary for the circulatory distribution of the thyroid hormone. It remains to be shown that such protein-binding controls the thyroidal metabolism of the tissues.

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THE ISOLATION OF Δ^4-PREGNENE-11β,17α,20α, 21-TETROL-3-ONE AND Δ^4-PREGNENE-11β,17α, 20β,21-TETROL-3-ONE FROM HUMAN ADRENAL INCUBATES

TO THE EDITOR:

Previous reports (1) from this laboratory concerning data obtained by toluene:propylene glycol paper chromatography of extracts of human adrenal slices incubated in autologous plasma indicated the regular occurrence of 3 or more poorly separated substances which were more polar than hydrocortisone. In the present study, paper chromatography was carried out as previously reported (1). Further separation of the region more polar than hydrocortisone was accomplished by rechromatography in the chloroform:formamide system, yielding 2 substances which, after characterization (Table 1), were shown to be Δ^4-pregnene-11β, 17α, 20α, 21-tetrol-3-one (F glycol-20α) and the 20β-isomer (F glycol-20β). The isolation of these glycols from adrenal tissue was first reported by Reichstein in 1936 (2).

Preliminary determinations showed that the average concentrations of F glycol-20α and F glycol-20β in the incubated tissue were 33 and 36 micrograms per gram respectively, compared to 278 micrograms per gram for hydrocortisone (3). The amount of the glycols increased 100 per cent or more upon addition of corticotropin to the incubation medium.

These results may indicate that the reduction of the 20-ketone producing the F-glycols isolated from urine (4–6) may not necessarily be entirely

TABLE 1. IDENTIFICATION OF GLYCOLS IN HUMAN ADRENAL INCUBATES

Procedure	F glycol-20α		F glycol-20β	
	Reference	Isolated	Reference	Isolated
1. R_{DOC} in toluene:propylene glycol	—	.0048	.006	.006
2. R_F* in chloroform:formamide	.52	.52	.69	.69
3. R_E* in benzene:formamide	.15	.145	.20	.19
4. R_{DOC} diacetate in toluene:propylene glycol	.4	.4	.17	.18
5. Blue tetrazolium reaction	Negative	Negative	Negative	Negative
6. Absorption maxima in methanol	240 mμ	240 mμ	240 mμ	240 mμ
7. Lead tetracetate test	+	+	+	+
8. Fluorescence color in sulfuric acid	Green	Green	Green	Green
9. Maxima activation and fluorescence spectra in sulfuric acid	460–510 mμ	460–510 mμ	460–510 mμ	460–510 mμ
10. Absorption maxima in sulfuric acid (mμ)	236 284 320 380 460 520	236 284 320 380 460 520	240 280 320 460	240 280 320 460
11. Oxidation with $NaBiO_4$ to 17-ketosteroid	+	+	+	+
12. Spectrum in H_2SO_4 of 17-ketosteroid (mμ)	289 380 400 460	289 380 400 460	289 380 400 460	289 380 400 460
13. Infrared spectrometry	See text			

* R_{DOC} = mobility with DOC taken as unity.
R_F = mobility with F taken as unity.
R_E = mobility with E taken as unity.

due to metabolism of endogenous hydrocortisone by the liver or kidney, but may actually be in part a function of adrenal metabolism.

Chromatography in the free form in 3 solvent systems and as the acetate in another system showed that the 2 compounds had mobilities similar to those of authentic samples (kindly supplied by Dr. Karl Pfister, Merck, Sharp and Dohme and Dr. Seymour Bernstein, Lederle Laboratories).

The reaction with blue tetrazolium for determination of alpha ketols was negative, showing the lack of this grouping or reducing substituent. However, the reaction with lead tetra-acetate, when performed on the chromatogram, yielded a white spot on a dark background, indicating adjacent carbons with oxygen functions. Oxidation of both substances with sodium bismuthate furnished a 17-ketosteroid having the same mobility as 11β-hydroxyandrostene-3,17-dione in methylcyclohexane:propylene glycol. This oxidation product had an absorption spectrum in sulfuric acid the same as that of 11β-hydroxyandrostene-3,17-dione. Separation of the 2 epimers occurred readily in chloroform:formamide, or as the acetates in toluene:propylene glycol.

The fluorescence and activation spectra of sulfuric-acid solutions of both epimers of the isolated F-glycols were the same as those presented by the reference compounds. The spectra were the same as that shown by cor-

ticosterone and hydrocortisone, although of lower intensity. Both the isolated substances and the reference steroids exhibited the characteristic green fluorescence in sulfuric acid. Both substances showed absorption maxima at 240 mμ in alcoholic solution, as did the reference steroids. The dissimilarity of the absorption spectra of the 20α and 20β epimers in concentrated sulfuric acid (Table 1) was sufficient to permit differentiation. The spectra obtained with the isolated steroids were the same as those obtained with the reference steroids under equivalent conditions.

The acetates were purified by chromatography on silica gel prior to infrared spectrometry. (Performed through the courtesy of Dr. T. F. Gallagher, Sloan Kettering Institute.)

The isolated compounds yielded infrared spectra practically identical to Atlas curves #189 and #513 (7), which provided further confirmation for the identifications. Due to lack of material, it was not possible to obtain sufficiently clear spectra on the double-beam infrared spectrophotometer, to differentiate between the 2 epimers.

Acknowledgment

This study was supported in part by Grants CY-3644 and A-1509 from the National Institutes of Health, U. S. Public Health Service.

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DEPRESSION OF THE SERUM CHOLESTEROL LEVEL BY TRIIODOTHYROPROPIONIC ACID

TO THE EDITOR:

The present interest in agents which lower the concentration of serum cholesterol arises from concepts currently under scrutiny in relation to the pathogenesis and treatment of atheromatosis. Depression of the serum cholesterol level has been one of the effects observed in association with the administration of certain acetic-acid analogues of thyronine, as well as desiccated thyroid, thyroxine and triiodothyronine (1–11). Triiodothyropropionic acid has been studied little, if at all, in this regard. Since this compound has been reported to be three hundred times more active than thyroxine in tadpole metamorphosis, and ten times less active in raising the metabolic rate of rats (12, 13), it was felt worth while to determine its influence upon the level of serum cholesterol in human subjects.

MATERIAL AND PROCEDURE

Four men, 65, 41, 62 and 65 years of age, without evidence of active disease but with varying degrees of arteriosclerosis, were selected from the wards of the Intermediate Service, VA Center. The ward routine, including the diet, was not changed. The diet contained roughly 2200 calories daily, including 90 grams of fat.

In all subjects, a control period of four and a half weeks was employed. During this time, 2 baseline serum cholesterol determinations (method of Sperry and Webb (14)) were made in each case. Among 20 total serum cholesterol determinations made in 2 patients prior to this study, a satisfactorily narrow fluctuation of ± 20 mg. per 100 ml. had been obtained. During the time of treatment, which lasted approximately three months, 2 to 3 cholesterol determinations were made in each patient at monthly intervals, and weekly for three weeks during the recovery period. Both total cholesterol and cholesterol esters were assayed in each instance. Daily blood pressures, pulse rates and weights were recorded during the whole course of the study. All patients were observed daily for any signs of thyrotoxicosis.

RESULTS

At the end of the initial five-week period of therapy with triiodothyropropionic acid in an oral dosage of 3 mg. daily in three fractional doses, the average total serum cholesterol levels had not dropped significantly except

in Patient III, in whom the value was 64 mg. per 100 ml. less than during the control period (Table 1). At this point the dosage was increased to 4 mg. and then to 6 mg. daily within the next two weeks by increasing the daily intake of triiodothyropropionic acid each week by 1 mg. After ingestion of 6 mg. of the drug per day for a period of two to four weeks, a significant depression of the total serum cholesterol level was observed in each of the 4 subjects; expressed in milligrams per 100 milliliters of serum, the drops were −43, −47, −106 and −61 respectively. During the twelfth week of therapy, after four to five weeks of ingestion of triiodothyropropionic acid in a dosage of 6 mg. daily, the levels of serum cholesterol in Patients I and II had decreased still further (Table 1). When the control level for serum cholesterol in each patient was fixed arbitrarily at 100 per cent, an average decrease of 25 per cent was observed by the twelfth week of the study. The individual decreases were 50, 29, 25 and 24 per cent.

TABLE 1. DECREASES IN SERUM CHOLESTEROL LEVEL IN 4 OLDER MEN RECEIVING FROM 3 TO 6 MG. OF TRIIODOTHYROPROPIONIC ACID DAILY

Week of test period	Daily dose of drug (mg.)	Total serum cholesterol (mg. per 100 ml.)							
		Patient I		Patient II		Patient III		Patient IV	
		Range & average	Change (mg./100 ml.) from control	Range & average	Change (mg./100 ml.) from control	Range & average	Change (mg./100 ml.) from control	Range & average	Change mg./(100 ml.) from control
Control	0	(247–268)* 258± 10.1**	—	(176–184) 180± 3.7	—	(209–216) 212± 9.7	—	(239–246) 242± 2.9	—
5	3 (5 wks.)	(258–268) 261± 4.5	+ 3	(155–173) 166± 7.5	−14	(138–159) 148± 7.1	−64	(206–213) 209± 2.6	−33
9†	6 (2–4 wks.)	(207–227) 215± 8.1	−43	(135–150) 143± 6.5	−47	(106–133) 127± 10.5	−85	(176–186) 181± 3.9	−61
						(98–113) 106± 6.6	−106		
12	6 (4–5 wks.)	(190–215) 194± 12.2	−64	(123–133) 128± 4.2	−52			(177–187) 183± 4.2	−59
13	0‡	(266–281) 271± 6.8	+13	(169–173) 170± 2.4	−10	(199–204) 202± 2.2	− 9	(197–207) 201± 4.8	−41
						(228–243) 236± 7.0	+24		
14	0	(271–281) 276± 4.2	+18	(189–195) 192± 2.6	+12	(227–233) 231± 2.0	+19	(243–249) 244± 2.6	+ 2

* Range.

** Average (2–3 determinations) and standard deviation.

† The drug intake had been increased after the fifth week by 1 mg. daily per week and was thus brought up to a maintenance dosage of 6 mg. daily.

‡ Patient IV was still taking 6 mg. of the drug daily. It was stopped the day after this cholesterol determination.

In only 1 case (Patient IV) was there an escape from the cholesterol-lowering effect of triiodothyropropionic acid during the time of its administration. In the other 3 cases, a rapid rise in the level of serum cholesterol (to and above the control value) occurred within seven to fourteen days following the cessation of treatment.

The esterification of cholesterol was remarkably constant percentagewise in all subjects, fluctuating narrowly between 69 and 73 per cent of the total cholesterol.

A basal metabolic rate was determined in each patient after treatment with 4 to 6 mg. of the compound daily for several weeks; the values were +3.5, +4.5, −1 and −6 per cent, respectively. There were no clinical signs of excessive stimulation of metabolism due to this thyroid hormone derivative, and no change in the pulse rate, blood pressure or body weight in all 4 patients throughout the entire period of observation.

SUMMARY AND CONCLUSIONS

It is clear from this study on 4 elderly men with arteriosclerosis that not only abnormally high levels but also normal levels of serum cholesterol can be depressed routinely by administration of triiodothyropropionic acid in an oral dosage of 3 to 6 mg. daily. Continued use of the drug will maintain such depressed cholesterol levels for at least twelve weeks, without signs of thyrotoxicosis.

The practical usefulness of triiodothyropropionic acid or certain of the other thyroxine analogues in maintaining a lowered serum cholesterol level without raising metabolic requirements remains unproved. Should their worth be established in this respect, it is far from clear that such compounds will be useful in the treatment or prevention of atherosclerotic lesions.

HANS G. BAUER, M.D.
THOMAS H. MCGAVACK, M.D.
LEON SWELL, PH.D.

The Intermediate Service,
Veterans Administration Center,
Martinsburg, West Virginia, and
The Department of Medicine,
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School of Medicine,
Washington, D. C.
September 8, 1958

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The Endocrine Society

THE 1959 ANNUAL MEETING

The Forty-First Annual Meeting of The Endocrine Society will be held in the Haddon Hall Hotel, Atlantic City, New Jersey, Thursday, Friday, and Saturday, *June 4, 5 and 6, 1959.*

The Chairman of Local Arrangements is Dr. Matthew Molitch.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily, and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 5, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Chalfonte-Haddon Hall Hotel will hold 300 bedrooms for members until May 1, 1959, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations now directly with the hotel, advising them of time and date of arrival and departure. Avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 1, 1959 to members whose current dues have been paid.

PROGRAM

THURSDAY, JUNE 4, 1959

8:00 A.M.—Registration—Stair Hall, Haddon Hall Hotel

I. 9:00 A.M.—THYROID

Carolina Room—Chalfonte

Chairmen: *J. E. Rall* and *W. Parson*

1. The Effect of Trypan Blue on Thyroid Function in the Rat.
   Takashi Yamada (introduced by Monte A. Greer).
2. The Effect of Excessive Iodide on the Thyroid of the Rat.
   Valerie A. Galton and *Rosalind Pitt-Rivers* (introduced by Sidney Ingbar).
3. Comparison of *in Vivo* and *in Vitro* Tissue Effects of Triiodothyronine.
   Roger L. Greif.
4. The Effect of Triiodothyropropionic Acid on Serum Cholesterol and Oxygen Consumption in Myxedema and Euthyroidism.
   Robert D. Leeper (by invitation), *Richard S. Benua, Soichi Kumaoka* (by invitation), *Jacob L. Brener* (by invitation) and *Rulon W. Rawson.*

3. Respiratory Stimulation in Young Dogfish by Thyroid Hormone.
Aubrey Gorbman and *A. W. Pritchard* (by invitation). (Invited paper—20 minutes.)

INTERMISSION

6. Idiopathic Familial Elevation of Thyroxine Binding Protein.
William H. Beierwaltes.
7. Metabolism of Thyroxine by Human Tissue Slices.
Edwin C. Albright and *Frank C. Larson* (by invitation).
8. Metabolic Regulation of Organic Iodinations in Thyroid Homogenates.
George C. Schussler (by invitation) and *Sidney H. Ingbar.*
9. Protein Iodination in Sheep Thyroid Mitochondrial-Microsomal Preparations.
Leslie J. DeGroot and *Evelyn Carvalho* (introduced by John B. Stanbury).
10. Persistent Thyroid Function after Hypophysectomy.
O. H. Pearson, A. G. Pazianos (by invitation), *E. Greenberg* (by invitation) and *R. S. Benua.*
11. Effect of Stimulation of the Cervical Sympathetic Nerve on the Thyroidal Release of I^{131}-Labeled Hormones.
Shiro Iino, Kazuo Shizume and *Shigeo Okinaka* (introduced by Monte A. Greer).

II. 2:00 P.M.—ADRENAL CORTICAL SYNDROMES—STEROID METABOLISM

Carolina Room—Chalfonte

Chairmen: *J. McArthur* and *A. Bongiovanni*

12. 6-Beta Hydroxylation of Cortisol; A Quantitatively Important Pathway of Metabolism in the Human Newborn.
E. Colle (by invitation), *R. A. Ulstrom, J. Burley* (by invitation) and *R. Gunville* (by invitation).
13. The Urinary Excretion of Three C-21 Steroids in the Adrenogenital Syndrome.
Alfred M. Bongiovanni, Walter R. Eberlein and *J. Darrel Smith* (by invitation).
14. The Renal Handling of 17-Hydroxycorticosteroids.
R. O. Morgen (by invitation), *A. J. Blair* (by invitation) and *J. C. Beck.*
15. The Influence of ACTH and Hypophysectomy on the Steroid Conjugating Capacity of the Liver.
David L. Berliner (by invitation), *Charles J. Nabors, Jr.* and *Thomas F. Dougherty.*
16. Direct and Indirect Estimation of Pituitary ACTH Reserves in Man Following Steroid Therapy.
Donald A. Holub (by invitation), *Julian I. Kitay, Andrew G. Frantz* (by invitation) and *Joseph W. Jailer.*
17. ACTH Suppression Tests in Patients with Cushing's Syndrome.
Grant W. Liddle.

INTERMISSION

18. Urinary Steroid Metabolites in Hypophysectomized Women During Adrenal Insufficiency and Initial Phases of ACTH Stimulation.
Hildegard Wilson, Mortimer B. Lipsett and *Lillian C. Butler* (by invitation).

19. Suppression of Urinary 17-Ketosteroids in Virilized Women by Estrogen Therapy.
Raymond C. Mellinger and *Richmond W. Smith, Jr.*
20. Transcortin: Characteristics and Levels in Various Conditions.
Avery A. Sandberg and *W. Roy Slaunwhite, Jr.*
21. Studies on the Mechanism of Elevation of Plasma 17-Hydroxycorticosteroids Induced by Estrogen Administration in Man.
Eleanor Z. Wallace and *Anne C. Carter.*
22. Endocrine Effects of Methylenedianiline.
William W. Tullner
23. Salt Losing Hormone in Hyperkaliemic Familial Periodic Paralysis.
R. Klein.

III. 2:00 P.M.—PARATHYROIDS: PITUITARY-ADRENAL SYSTEM

Viking Room—Haddon Hall

Chairmen: *G. D. Whedon* and *D. Nelson*

24. Isolation of Parathyroid Hormone Following Extraction with Phenol.
G. D. Aurbach.
25 Purification of Parathormone B by Countercurrent Distribution.
Howard Rasmussen.
26. Activation of Parathyroid Hormone by Cysteine.
Alexander D. Kenny.
27. Effect of Parathyroid Extract on Bone Matrix and Seromucoid.
William P. Deiss and *Leila B. Holmes* (by invitation).
28. Further Evidence for a Dual Effect of Parathyroid Hormone.
John J. Canary (by invitation), *H. Allan Bloomer* (by invitation), *Gabriel G. Carreon* (by invitation) and *Laurence H. Kyle.*
29. ACTH Secretion in Rats with Hypothalamic Lesions.
A. Brodish.

INTERMISSION

30. Limbic System Influences on the Pituitary-Adrenal Cortical System.
John W. Mason, Walle J. H. Nauta (by invitation), *Joseph V. Brady* (by invitation) *and James A. Robinson* (by invitation).
31. Effect of Diencephalic Stimulation on Adrenocortical and Adrenal Medullary Secretion in the Dog.
William H. Ganong and *Alan Goldfien.*
32. Adrenal Cortical and Medullary Responses to Trauma in Dogs with Isolated Pituitaries.
Richard H. Egdahl (introduced by D. M. Hume).
33. The Early and Late Effects of Hypothalamic Destruction or Removal on Pituitary-Adrenal Activity.
David M. Hume and *Benjamin F. Jackson* (by invitation).
34. Ion-Exchange Chromatography of Pituitary Preparations.
Andrew V. Schally (by invitation) and *Roger Guillemin.*
35. ACTH Releasing Factor Active in the Guinea Pig Test.
M. Sideman (by invitation) and *H. Sobel.*

FRIDAY, JUNE 5, 1959

8:30 A.M.—Registration—Stair Hall, Haddon Hall Hotel

IV. 9:00 A.M.—ANTERIOR PITUITARY

Carolina Room—Chalfonte

Chairmen: *E. Knobil* and *F. L. Engel*

36. The Cells of the Human Adenohypophysis in Adrenal Cortical and Thyroid Disorders.
Calvin Ezrin and *Heidi Swanson* (introduced by R. F. Farquharson).
37. A Method for the Quantitative Biological Assay of ACTH in Blood.
Paul L. Munson, Wolfgang Toepel (by invitation) and *Mindel C. Sheps.*
38. Increase in Sugar Permeability in the Rat Adrenal Produced by ACTH.
J. Eichhorn, I. D. K. Halkerston, M. Feinstein and *O. Hechter* (by invitation).
39. Contrasting Requirements for the Lipolytic Action of Corticotropin and Epinephrine on Adipose Tissue *in Vitro.*
J. Earle White (by invitation), *Enrique Lopez* (by invitation) and *Frank L. Engel.*
40. Evidence for Species Specificity of Human, Beef and Rat ACTH Demonstrated by Assay *in Vitro.*
Julian I. Kitay, Donald A. Holub (by invitation) and *Joseph W. Jailer.*
41. A Comparative Immunological Investigation of Pituitary Growth Hormone from Various Species.
T. Hayashida (by invitation) and *C. H. Li.*

INTERMISSION

42. Growth-Stimulating and Other Effects of Human Growth Hormones (Li) in a Pituitary Dwarf.
Roberto F. Escamilla, John J. Hutchings (by invitation), *William C. Deamer* (by invitation) and *Choh Hao Li.*
43. Endocrine Function in Acromegaly.
George J. Hamwi and *Thomas G. Skillman* (by invitation).
44. The Influence of Hypophysectomy and Growth Hormone on Oxidation of Palmitate-1-C^{14} by the Rat.
M. J. Franklin (by invitation) and *E. Knobil.*
45. Unesterified Fatty Acid Oxidation Rates in Acromegaly, Myxedema, Hyperthyroidism and Diabetes.
Josiah Brown and *Leslie R. Bennett* (by invitation).
46. Evidence for Direct Action of Growth Hormone (GH) on Red Cell Formation.
Albert S. Gordon, Burton S. Dornfest (by invitation) and *Anthony Crusco.*
47. Factors Involved in Production of Suspensions of Free Cells Derived from Hypophyses of Rats.
Griff T. Ross (by invitation) and *Robert C. Bahn.*

1:30 P.M. ANNUAL BUSINESS MEETING, CAROLINA ROOM, CHALFONTE

V. 2:30 P.M.—ESTROGENS AND ANDROGENS

Carolina Room—Chalfonte

Chairmen: *R. Huseby* and *L. L. Engel*

48. The Isolation and Identification of Estrogens from Peripheral Blood with a Practical Method of Analysis.
Georg W. Oertel (by invitation), *Charles D. West* and *Kristen B. Eik-Nes.*
49. "Free Estriol" in Blood Plasma.
Joseph C. Touchstone and *John W. Greene, Jr.* (by invitation).
50. Effect of Chorionic Gonadotropin (HCG) on Conversion of Estradiol to Estriol by Perfused Human Placenta.
Philip Troen.
51. Physiologic and Biochemical Differences between the Estrogen-Sensitive Transhydrogenase and Estradiol Dehydrogenase.
C. A. Villee, D. D. Hagerman (by invitation) and *J. M. Spencer* (by invitation).
52. The Inhibition of Placental Steroid Mediated Transhydrogenase by 2′-Adenylic Acid.
Nina Hollander (by invitation), *Joseph D. Brown* (by invitation) and *Vincent P. Hollander.*
53. Adrenal Estrogens: *in Vivo* Conversion of Cortisone to Phenolic Estrogens.
Edward Chang (by invitation) and *Thomas L. Dao.*

INTERMISSION

54. Estrogen Synthesis in a Male Patient with a Feminizing Adrenocortical Carcinoma and Hypertension.
C. D. West, L. F. Kumagai (by invitation), *E. L. Simons* (by invitation) and *P. E. Wall* (by invitation).
55. The Effect of Estrogen on Steroid Levels in Plasma and Urine.
W. L. Herrmann, I. Schindl (by invitation), and *P. K. Bondy.*
56. The Conversion of Progesterone-4-C^{14} to 21-Hydroxylated Steroids by Normal Rat Testis Stimulated with Human Chorionic Gonadotropin.
Hernan F. Acevedo (by invitation), *Oscar V. Dominguez* and *Leo T. Samuels.*
57. Steroid 21-Hydroxylase in Mouse Testicular Interstitial Cell Tumors.
Oscar V. Dominguez, Robert A. Huseby and *Leo T. Samuels.*
58. Studies of Structure-Function Relationships of Steroids: Anabolic Potency of Some Testosterone Analogs.
Herbert A. Burke, Jr. (by invitation) and *Grant W. Liddle.*
59. Influence of Androgen on Prostatic Protein Synthesis.
Wells E. Farnsworth (introduced by Grosvenor W. Bissell).

VI. 2:30 P.M.—PITUITARY HORMONES—NEUROENDOCRINE MECHANISM

Viking Room—Haddon Hall

Chairmen: *J. Everett* and *A. Gorbman*

Those registered for the meeting of the American Diabetes Association will be admitted to this meeting.

60. Lithosperm Inhibition of Blood Pressure Depressor Effect of Oxytocin in the Fowl.

W. R. Breneman, Marvin Carmack, Daniel Overack and *Richard Shaw* (introduced by Aubrey Gorbman).

61. PLASMA AND PITUITARY LH: DETECTION AND QUANTITATIVE BIOASSAY IN THE RAT FOLLOWING GONADECTOMY.
Albert Parlow (introduced by R. O. Greep).
62. USE OF THE RED EFT DIEMICTYLUS VIRIDESCENS IN THE ASSAY OF PROLACTIN.
William C. Grant, Jr. (introduced by Aubrey Gorbman).
63. EFFECTS OF CAPTIVITY ON PRODUCTION OF GONADOTROPHINS IN PINTAIL AND BLACK DUCKS AND RESPONSE TO EXOGENOUS HORMONES.
R. E. Phillips and *A. van Tienhoven* (introduced by S. L. Leonard).
64. OVULATION IN THE DOMESTIC FOWL.
R. D. Fraps. (Invited paper, 20 minutes).

INTERMISSION

65. OVULATION AFTER STIMULATING THE RAT HYPOTHALAMUS LATE IN DIESTRUS.
J. W. Everett, R. L. Riley (by invitation) and *C. D. Christian* (by invitation).
66. DEVELOPMENT OF PERMANENT ABNORMAL CYCLES OF BEHAVIOR IN RATS AFTER EXPERIMENTAL PITUITARY DAMAGE.
Curt P. Richter.
67. HYPOTHALAMUS AND GROWTH.
S. Reichlin.
68. TSH "REBOUND PHENOMENON" IN RATS AFTER GOITROGEN WITHDRAWAL.
S. A. D'Angelo.
69. PITUITARY HORMONE PRODUCTION AND RELEASE IN THE THYROIDECTOMIZED RAT AFTER THYROXIN ADMINISTRATION.
A. N. Contopoulos, M. E. Simpson (by invitation) and *A. A. Koneff* (by invitation).
70. SUBCORTICAL REGIONS OF THE PRIMATE BRAIN REGULATING RELEASE OF VASOPRESSIN FROM THE NEUROHYPOPHYSIS.
Joseph F. Dingman, Eduardo Gaitan (by invitation), *Akira Arimura* (by invitation) and *Robert G. Heath* (by invitation).

VII. ANNUAL DINNER—Haddon Hall Hotel

E. Perry McCullagh, President, presiding.

6:30 P.M. Cocktails—The English Lounge.
Sponsored by the donors of the Awards of the Society:
Ciba, Ayerst, Schering, Upjohn and Squibb. Attendance limited to holders of dinner tickets.

7:30 P.M. Dinner—The Vernon Room.
Presentation of the 1959 Awards of the Society by *CharlesKochakian:*
The Fred Conrad Koch Award
The Ciba Award
Ayerst Fellowship
Schering Scholar
Upjohn Scholar
President's Address: *Dr. McCullagh*

SATURDAY, JUNE 6, 1959

8:30 A.M. Registration—Stair Hall, Haddon Hall Hotel.

9:00 A.M. Opening scientific session of The American Diabetes Association will be held at The Haddon Hall Hotel in The Vernon Room. Endocrine Society Badge will admit you to this meeting.

VIII. 9:00 A.M.—ADRENAL GLAND

Carolina Room—Chalfonte

Chairmen: *M. Grumbach* and *F. C. Bartter*

71. STIMULATORY EFFECT OF ALDOSTERONE AND CORTISOL ON THE WORK OF THE ISOLATED RAT HEART-LUNG.
Kathryn W. Ballard (by invitation) and *George Sayers.*
72. THE HEMODYNAMIC PARAMETER MEDIATING INCREASE IN ALDOSTERONE SECRETION.
Donald S. Gann (by invitation), *Ivor H. Mills* (by invitation) and *Frederic C. Bartter.*
73. PRELIMINARY CHARACTERIZATION OF GLOMERULOTROPIN.
Gordon Farrell, Avery D. Pratt, Jr. (by invitation), *Laurence S. Harris* (by invitation) and *Robert C. Banks* (by invitation).
74. CHANGES IN METABOLIC ACTIVITIES OF STEROID HORMONES ASSOCIATED WITH METHYLATION AND HYDROXYLATION IN RING D.
Maurice M. Pechet, Evelyn L. Carroll (by invitation) and *Anne P. Forbes.*
75. EFFECT OF WALKER CARCINOMA ON STEROID DIABETES IN RAT.
David J. Ingle (introduced by Dwight J. Ingle).
76. ACUTE EFFECTS OF ADRENALECTOMY AND HYDROCORTISONE ON GLUCOSE TOLERANCE OF DIABETIC AND EVISCERATE RATS.
O. K. Smith and *C. N. H. Long.*

INTERMISSION

77. INFLUENCE OF AGE, ADRENALECTOMY AND CORTISOL ADMINISTRATION ON THE ACTIVITY OF HEPATIC GLUTAMIC-PYRUVIC TRANSAMINASE.
Homer Harding, Fred Rosen and *Charles Nichol* (introduced by Avery A. Sandberg).
78. THE METABOLISM OF 2-C^{14} PYRUVATE IN NORMAL AND ADRENALECTOMIZED RATS.
William W. Winternitz.
79. OBSERVATIONS ON THE ROLE OF THE ADRENAL GLAND IN THE METABOLIC RESPONSE TO SURGERY.
Maureen Smith, H. A. F. Dudley, J. S. Robson and *C. P. Stewart* (introduced by Grant W. Liddle).
80. RENAL-CARDIOVASCULAR PATHOLOGY IN AGING RATS WITH AND WITHOUT EXPOSURE TO ACTH AND STRESSORS.
George F. Wilgram (by invitation) and *Dwight J. Ingle.*
81. THE EFFECTS OF ANOXIA ON ADRENAL CORTICAL AND MEDULLARY SECRETION IN THE DOG.
Alan Goldfien and *William F. Ganong.*
82. EFFECT OF ADRENALECTOMY AND CORTISONE ON RATE OF ERUPTION OF MAXILLARY INCISORS IN FEMALE RATS.
L. V. Domm, W. A. Wellband (by invitation) and *J. P. Howard* (by invitation).

IX. 2:00 P.M.—INSULIN AND DIABETES

Carolina Room—Chalfonte

Chairmen: *S. Fajans* and *A. Renold*

83. Differential Resin Binding of Insulin in Sera from Insulin Responsive and Resistant Diabetic Subjects.
Marvin L. Mitchell and *Mary E. O'Rourke* (by invitation).
84. Serum Insulin-Like Activity in Patients with Pancreatic Islet-Cell Tumors.
Yves M. Dagenais (by invitation), *Albert E. Renold, Donald B. Martin* (by invitation) and *Vilma Lauris* (by invitation).
85. Role of Individual Organs in Carbohydrate Metabolism in Intact Normal Dogs.
Dorothy H. Henneman, William Shoemaker (by invitation) and *William Walker* (by invitation).
86. Hepatic Glucose Metabolism in Two Forms of Spontaneous Hyperglycemia.
Gaston R. Zahnd, Jean Christophe and *George Yerganian* (introduced by Albert E. Renold).
87. Carbohydrate Metabolism in Amphibians and Reptiles.
Malcolm R. Miller (introduced by Frank L. Engel). (Invited paper, 20 minutes.)

INTERMISSION

88. On the Mechanism of the Blood Glucose Lowering Action of Insulin.
N. Altszuler, A. Dunn (by invitation), *R. Steele, D. T. Armstrong* (by invitation), *J. S. Bishop* (by invitation) and *R. C. de Bodo.*
89. An Insulin Effect on Peptide Synthesis Independent of Glucose or Amino Acid Transport.
Ira G. Wool and *M. E. Krahl* (by invitation).
90. Comparison of Epinephrine and Insulin on Adipose Tissue Metabolism.
George F. Cahill, Jr., Bernard Leboeuf (by invitation) and *Robert Flinn* (by invitation).
91. The Action of Insulin, Tolbutamide, and Hypoglycin A on the Adipose Tissue of Rats.
Thomas C. Smith, Lena Will (by invitation) and *Ira Ringler.*
92. Relation of Insulinogenesis to Pancreatic B Cell Acid and Glucose-6-Phosphatase.
Sydney S. Lazarus (introduced by Bruno W. Volk).
93. Effect of Glucagon on Hepatic Blood Flow and Metabolism in Unanesthetized Dog.
W. C. Shoemaker and *T. B. Van Itallie* (introduced by Jay J. Gold).

TO BE READ BY TITLE

(Abstracts 94–170)

THE 1959 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

During the past year a substantial legacy has been bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is to be the highest honor of The Endocrine Society and is to be represented by a medal that is to be known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award will replace the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr.

Monte A. Greer. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street,

Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

ELEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE AND DEPARTMENT OF CONTINUING MEDICAL EDUCATION

SAN FRANCISCO, CALIFORNIA

November 9 through 13, 1959

For information concerning the program and registration, write to *Seymour M. Farber, M.D.*, Assistant Dean in charge of Continuing Medical Education, University of California Medical Center, San Francisco 22, California.

The registration fee is $100. Residents and Fellows will be admitted for a reduced fee of $30. Those wishing to reserve rooms should communicate directly with the Whitcomb Motor Hotel, 8th and Market St., San Francisco.

The American Goiter Association

THE 1959 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Drake Hotel, Chicago, Illinois, on *April 30, May 1 and 2, 1959.* Secretary, John C. McClintock, M.D., 149½ Washington Ave., Albany 10, N. Y.

PRELIMINARY PROGRAM

THE DRAKE, CHICAGO, ILLINOIS

April 30, May 1 and 2, 1959

Thursday, April 30, 1959—8:30 A.M.

Urinary I^{127} Excretion and the T_3 Suppression Test as Aids in the Diagnosis of Thyroid Disorders.

David H. Solomon, M.D. and Delores E. Johnson, M.D., Torrance, California.

Inhibition of Thyroidal Iodide Concentrating by Triiodothyronine: Evidence for an Effect not Due to Depression of Thyrotrophin Production but Dependent on the Pituitary.

N. S. Halmi, M.D., D. K. Granner, B.A., H. Albert, B.A. and *D. J. Doughman, B.M.E.*, Iowa City, Iowa.

Effect of Thyroxine Analogues on a Mouse Pituitary Transplantable Tumor.

Soichi Kumaoka, M.D., William L. Money, Ph.D. and *Rulon W. Rawson, M.D.*, New York, N. Y.

Iodine Metabolism in Hydatidiform Mole and Choriocarcinoma.

J. Thomas Dowling, M.D., Norbert Freinkel, M.D., and *Sidney H. Ingbar, M.D.*, Los Angeles, California and Boston, Massachusetts.

The Occurrence of Iodinated Tyrosines in Human Plasma.

Helen E. A. Farran, B.Sc. and *R. Bird, Ph.D.*, London, England.

Preparation and Metabolism of Iodinated Oligopeptides.

C. Beckers, M.D., M. J. Spiro, Ph.D. and *J. B. Stanbury, M.D.*, Boston, Massachusetts.

Iodide Goiter.

Jaime Paris, M.D., William M. McConahey, M.D., Charles A. Owen, Jr., M.D., Lewis B. Woolner, M.D. and *Robert C. Bahn, M.D.*, Rochester, Minnesota.

Oxygen Consumption and Thyroxine Deiodination by Human Leukocytes.

G. S. Kurland, M.D., M. V. Krotkov, M.D. and *A. S. Freedberg, M.D.*, Boston, Massachusetts.

Thursday, April 30, 1959—2:00 P.M.

Transplacental Transfer of Antibodies to Thyroglobulin—An Etiology of Athyrotic Cretinism?

R. M. Blizzard, M.D., R. W. Chandler, M.Sc., B. H. Landing, M.D., J. M. Sutherland, M.D., V. M. Esselborn, M.D., G. J. Hamwi, M.D., M. Pettit, R. N., G. M. Guest, M.D. and *T. G. Skillman, M.D.*, Columbus, Ohio and Cincinnati, Ohio.

Thyroid Function of Adolescents Evaluated by Serum Butanol Extractable Iodine.

Evelyn B. Man, Ph.D., Frederic M. Blodgett, M.D. and *Andrew J. McGowan, Jr., M.D.*, New Haven, Connecticut.

Observations Concerning the Free Myo-Inositol of Thyroid Tissue.

Norbert Freinkel, M.D., Rex M. C. Dawson, Ph.D., Sidney H. Ingbar, M.D., Charles J. Goodner, M. D. and *Robert W. White*, Boston, Massachusetts, and Babraham, Cambridge, England.

"Metabolic Insufficiency": A Double Blind Study Using Triiodothyronine, Thyroxine, and a Placebo: With a Psychometric Evaluation of the Hypometabolic Patient.

Marvin E. Levin, M.D., St. Louis, Missouri.

Uric Acid Metabolism as Related to Thyroid Function.

Robert D. Leeper, M.D., Richard S. Benua, M.D., Jacob L. Brener, M.D. and *Rulon W. Rawson, M.D.*, New York, N. Y.

Depression of Serum Cholesterol in Myxedematous Patients by Doses of Sodium Dextro-Thyroxine which Have No Effect on the Basal Metabolic Rate, or Electrocardiogram.

Paul Starr, M.D., Los Angeles, California.

The Pulmonary Abnormalities in Myxedema.

William R. Wilson, M.D. and *George N. Bedell, M.D.*, Iowa City, Iowa.

A 30-Year Follow-Up Study of Thyroidectomy for Diffuse Exophthalmic Toxic Goiter.

Robert W. Bartlett, M.D., St. Louis, Missouri.

Friday, May 1, 1959—8:30 A.M.

Thyroxine Metabolism in the Presence of Thyroid Blocking Agents.

Sanford L. Jones, M. S. and *L. Van Middlesworth, Ph.D.*, Memphis, Tennessee.

Inhibition of the Conversion of Monoiodotyrosine to Diiodotyrosine by Propylthiouracil.

D. Ward Slingerland, M.D. and *Philip F. Mulvey, Jr., Ph.D.*, Boston, Massachusetts.

Selective Inhibition of the Calorigenic Activities of Certain Thyroxine Analogues with Chronic Thiouracil Treatment in Rats.

Neil R. Stasilli, Robert L. Kroc, Ph.D. and *Ray Edlin, B.S.*, Morris Plains, N. J.

Response of the Thiocyanate Blocked Gland to Thyroid Stimulating Hormone.

M. L. Mitchell, M.D. and *M. E. O'Rourke, B.A.*, Boston, Massachusetts.

The effect of BAL on Thyroid Function.

James V. Current, A.B., Ian B. Hales, M.B., B.S. and *Brown M. Dobyns, M.D., Ph.D.*, Cleveland, Ohio.

Thyroid Proteins in Nodular Goiter.

Leslie J. DeGroot, M.D., Boston, Massachusetts.

Iodinated Compounds in Thyroid Disturbances, Observed in Autoradiograms.

William A. Reilly, M.D., K. G. Scott, Ph.D., G. L. Searle, Ph.D. and *J. N. Castle, A.B.*, San Francisco, California.

An Objective Psychological Assessment of the Thyrotoxic Patient and His Response to Treatment: A Preliminary Report.

Leonard R. Robbins, M.D. and *David B. Vinson, Ph.D.*, Houston, Texas.

Friday, May 1, 1959—2:00 P.M.

PRESIDENTIAL ADDRESS:

Warren H. Cole, M.D., Chicago, Illinois.

VAN METER PRIZE ESSAY.

ROUND TABLE DISCUSSION OF THYROID CARCINOMA: MORPHOLOGIC TYPES AND THEIR BIOLOGIC BEHAVIOR.

Moderator:

Lewis B. Woolner, M.D., Rochester, Minnesota.

Participants:

John B. Hazard, M.D., Cleveland, Ohio.
G. H. Klinck, M.D., Washington, D. C.
Theodore Winship, M.D., Washington, D. C.

Saturday, May 2, 1959—8:30 A.M.

The Metabolism of Triiodothyronine in Graves' Disease.

Ian B. Hales, M.B., B.S. and *Brown M. Dobyns, M.D., Ph.D.*, Cleveland, Ohio.

Thyroidectomy for Hyperthyroidism Following Failure to Respond to Radioiodine Therapy.

Elmer C. Bartels, M.D., Boston, Massachusetts.

Thyroid Cancer in the Syrian (Golden) Hamster.

Joseph G. Fortner, M.D., Phillis A. George, F.R.C.S. and *Stephen S. Sternberg, M.D.*, New York, N. Y.

Serial Measurements of Exophthalmos in Graves' Disease: A 20-Year Study.

Willard Bartlett, M.D., St. Louis, Missouri.

Hyperthyrodism and Carcinoma of the Thyroid.

D. F. Federman, M.D., L. J. DeGroot, M.D., J. E. Dumont, M.D. and *J. B. Stanbury, M.D.*, Boston, Massachusetts.

Occult Papillary Carcinoma of the Thyroid Gland.

Lewis B. Woolner, M.D., Oliver H. Beahrs, M.D., B. Marden Black, M.D. and *F. Raymond Keating, Jr., M.D.*, Rochester, Minnesota.

Multiple Foci of Papillary Adenocarcinoma of the Thyroid. Influence of Treatment.

B. Marden Black, M.D., T. Allen Kirk, Jr., M.D., and *Lewis B. Woolner, M.D.*, Rochester, Minnesota.

Is There a Place for a Modified Neck Dissection in Surgery for Thyroid Carcinoma?

Melvin A. Block, M.D. and *J. Martin Miller, M.D.*, Detroit, Michigan.

TRAINING PROGRAM FOR STEROID BIOCHEMISTRY

The fourth session of the Training Program for Steroid Biochemistry sponsored by the U. S. Public Health Service will begin *October 1, 1959.* Applications are invited at the postdoctoral level for a one-year tenure with a stipend of $5500. The Program consists of lectures, planned laboratory, and eight months of research at the Worcester Foundation for Experimental Biology and Clark University or the University of Utah. Inquiries should be addressed to William R. Nes, Department of Chemistry, Clark University, Worcester, Massachusetts, or to Kristen Eik-Nes, College of Medicine, University of Utah, Salt Lake City. A predoctoral program also will be carried out in Worcester with stipends of $1800 for six months. *All applications must be received prior to June 1, 1959.*

18TH CONGRESS OF THE FEDERATION OF THE SOCIETIES OF GYNECOLOGY AND OBSTETRICS OF THE FRENCH LANGUAGE

The 18th Congress of the Federation will be held in *Paris, June 15–17, 1959.* All authors desiring to participate in the program should submit their abstracts *before May 15, 1959,* to the Secretary General, Dr. Maurice Robey, 4 Av. Saint-Honoré-d'Eylau, Paris 16, France. Applications for registration (12,000 francs) and information should be addressed to Dr. Suzor, Treasurer, 12 Square Henri Pate, Paris 16.

INTERNATIONAL CONGRESS ON IRRADIATION OF ENDOCRINE ORGANS

The First International Congress on Irradiation of Endocrine Organs will take place in Amsterdam, The Netherlands, *May 30–June 1, 1959.* For information, write to the Congress Office, c/o Dr. Johanna M. van Went, Banstraat 30, hoek de Lairessestraat, Amsterdam Z I, Nederland.

CORRECTION

In the article by Abelson, D. and Borcherds, D. A. entitled "Plasma Cortisol Levels in Man Following Oral and Intravenous Administration of Cortisol" (*J. Clin. Endocrinol. & Metab.* **19**: 219–223 (Feb.) 1959) the graphs and data that are above the legends for Figures 1 and 2 should be transposed.